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How pituitrin inhibits urine formation. E. F. ADOLPH. Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

In intact frogs (*Rana pipiens*) the rate of water intake through the skin was increased by subcutaneous injection of posterior pituitary extracts. If 2 to 9 units per kilogram were given, urine formation continued at normal rates or at rates that relieved the body only slowly of the extra water load; and when additional water was injected into the body, the urine rates were slightly increased, as they are in control frogs. If 60 to 200 units per kilogram were injected, extreme oliguria persisted for 2 hours; urine formation was then not promoted by water injection. The large dose was necessary in order that urine formation should be inhibited for as long as 2 hours, which period is required to measure urine output.

In pithed frogs, the outflow of urine from the ureter was observed simultaneously with the renal blood flow and the heart rate. Injection of pituitary extracts (free of chloretone) either subcutaneously or intravenously produced anuria in 6 of 8 experiments, and extreme oliguria in the other 2 experiments (0.8 to 11 units/kgm.). The anuria persisted for 10 to 45 minutes, depending on the dose, and was sometimes followed by polyuria. In one experiment, 90 units per kilogram stopped urine production for 3 hours. The flow of blood ceased in all glomeruli during the anurias; an increased rate of heart beat continued and the glomerular flow was fully restored during the polyurias.

The diuretic effect, therefore, is accompanied by marked improvement of the general circulation. The antidiuretic action of pituitary substance is localized in the afferent arterioles of the glomeruli; it is well known that cessation of circulation in them invariably yields anuria, and other structures of the kidney are then allowed no share in preventing urine excretion.

Total plasmapheresis. WILLIAM R. AMBERSON, JOHN STANBURY (by invitation), and EDNA WARWEG (by invitation). Department of Physiology, College of Medicine, University of Tennessee, and Marine Biological Laboratory, Woods Hole, Mass.

The method of plasmapheresis has been used by many investigators to secure reduction in plasma constituents without removal of red cells.

Extending the observations of others we have found it possible to remove practically all of the normal plasma from the blood stream of cats and dogs under ether anesthesia. We bleed from, and re-inject into, the carotid artery. The infusion fluid is 6 per cent gum-saline in which washed corpuscles of another animal of the same species are suspended (25 to 40 per cent). By repeated bleedings and the injections we pass large quantities of fluid through the body (300 to 400 cc. per kilo) until the blood proteins are reduced to traces of 0.1 to 0.2 per cent only. The blood pressure is maintained. Neither shock nor edema appear. The animals recover consciousness quickly and live indefinitely.

We have followed the rate of regeneration of the plasma proteins in cats and dogs after such total plasmapheresis. In cats the total protein value reaches the original normal in about 100 hours; in dogs recovery takes about 200 hours. The protein fractions have also been followed. Animals may survive a second total removal of their blood plasma after recovery from a first operation.

Gum-acacia is obviously able to maintain the water balance of the mammalian body in the nearly complete absence of its normal blood colloids. These are therefore seen to have mainly a physical significance, in maintaining the colloidal osmotic pressure of the blood, rather than a chemical influence upon the walls of the blood vessels.

Nitrogen partition of tissues after hypophysectomy and composition of weight loss. GILBERT B. AYRES (by invitation) and MILTON LEE. Harvard Medical School, Boston.

Both hypophysectomized and normal litter mate control rats kept on a paired-feeding regimen lose weight steadily, because the rate of food consumption is set by the operated animals at a level which is below maintenance for either group. The hypophysectomized rats lose a greater proportion of their initial body weight than do their control mates. The excess loss is greatest in the first few days after operation. The character of the weight lost is markedly different in the two groups. The controls lose a large proportion of their body fat stores but retain their original nitrogen content, or even gain in nitrogen. The hypophysectomized rats retain their original amounts of stored fat, but lose large amounts of nitrogen. They are apparently able to utilize ingested fat. The non-protein nitrogen partition of the tissues shows higher values in hypophysectomized rats for total N.P.N., urea, NH_3, creatine, and uric acid; and lower values for creatinine and glutathione. These changes probably indicate increased breakdown of tissues.

Effect of epinephrine administration on glucose excretion in depancreatized dogs. W. H. BACHRACH (by invitation), W. B. BRADLEY (by invitation), and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

During the course of some work on carbohydrate metabolism it was deemed advisable to repeat the work reported by Chaikoff and Weber on the source of the extra glucose yield of diabetic dogs given epinephrine.

Fourteen depancreatized dogs, fed a high carbohydrate diet covered by insulin until complete healing of the incision, were starved for three days. Twelve hour urine specimens were obtained by catheterization for the two twelve hour periods of the third day of starvation, and for the following twelve hour period during which epinephrine in one milligram doses was

administered at three hour intervals. Total nitrogen and glucose were determined on each specimen.

The average extra glucose excreted upon administration of epinephrine was 13.2 grams. The average total possible source of glucose (glycogen storage and gluconeogenesis from glycerol and protein), using figures determined by Chaikoff and Weber, was 25.8 grams. If muscle glycogen is excluded as a source of glucose, 11.9 grams of this extra glucose can be accounted for. From these figures it would appear unnecessary to postulate gluconeogenesis from fatty acids in such an experiment, a conclusion which confirms that of Bollman, Mann and Wilhelmj, who performed similar experiments.

The effect of diets high and low in calcium and phosphorus on the heart rhythm of young rats. ANNA M. BAETJER. Department of Physiology, Johns Hopkins School of Hygiene and Public Health.

Young rats were placed on adequate diets containing low, normal or high concentrations of calcium combined with a low or high phosphorus content. Electrocardiograms of the unanesthetized rats were taken weekly.

All low calcium diets caused a marked fall in heart rate which reached a permanently low level in 2 to 4 weeks. This was not due to vagal hypertonia since it was not abolished by complete atropinization. The slowed heart rate was accompanied by a marked failure of these rats to gain weight. Young rats, which failed to gain on a normal but quantitatively restricted diet, showed a similar though much more gradual decrease in rate. When viosterol was added after several weeks to a diet having a high calcium-low phosphorus content, thus causing a rapid temporary fall in blood calcium, there was an immediate decrease in heart rate.

Young rats on a high calcium diet showed, if anything, only a slightly slower heart rate than the controls.

Arrhythmias in the heart beat occurred among those rats fed a high calcium, low phosphorus (rachitogenic) diet, which according to previous workers leads to normal blood calcium but low blood phosphorus. The arrhythmias consisted in marked variations in sinus rhythm, variation in the P-R interval during the taking of the electrocardiogram, frequent failure of the ventricle to follow the auricular beat, usually preceded by lengthening of the P-R interval, and later partial and complete heart block, occurring in 40 per cent of the rats. The P-R interval did not, however, increase progressively throughout the weeks. The P wave was frequently difficult to determine or of irregular form indicating abnormal conduction in the auricles. The QRST complex was usually normal. Since these irregularities were temporary they must be of nervous origin, probably stimulated by handling the rats, and due either to a greater irritability of the nervous system or a more irritable response of the heart. These arrhythmias were not due to a moribund condition since the rats usually survived one or more such attacks. These arrhythmias were not found among rats placed on exactly similar diets but having a low calcium content.

Studies on the coagulability of blood and blood plasma using a photoelectric cell. EDWARD J. BALDES and K. K. NYGAARD (by invitation). Divisions of Biophysical Research and Surgery, The Mayo Foundation and Mayo Clinic, Rochester, Minnesota.

Using the coagelometer which consists essentially of a photronic cell

connected to a sensitive electrical instrument, the absorption of light has been studied during the process of the coagulation of specially prepared blood plasma obtained by a method previously described. In order to record the changes of the transmission of light in the solutions under observation the coagelgraph was devised and the study extended thereby to include measurements on the coagulation of whole blood.

The coagelgram indicates the time relations involved in the process of coagulation. During the first phase there is little or no change in light transmission. The second phase is characterized by a decrease in light transmitted, this portion of the coagelgram being represented often by a logistic function. The third phase is termed the rest period and is followed by the clot retraction period, the latter phase not being always obtainable.

Application of the method has been made clinically to the study of the conditions in which a disturbance of the coagulability of blood plasma is suspected. In severe obstructive jaundice, in thrombocytopenic purpura and in hemophilia the second phase of coagulation is prolonged while in certain cases of postoperative thrombosis a shortening of this period has been recorded.

The relation of total tension in isometric muscular contraction to initial tension and length of the muscle. M. GARCIA BANUS, A. ZETLIN (by invitation) and D. H. GERSH (by invitation). Department of Physiology, Tufts College Medical School.

Experiments were performed in decerebrated cats and frogs using a rigid isometric myograph of the non-friction, reflecting-mirror type. With a reflected beam of 2,000 mm. amplifications of as much as 500 times were obtained. The length of the muscle was changed by sliding the support to which the animal was fixed, the length being measured to within one tenth of a millimeter. Initial tension was varied either by changing the muscle length, or in the case of the decerebrated cat by different intensities of proprioceptor tonus. Both maximum short tetani and maximum single break stimuli were used. In all cases except the sartorius of the frog the stimulation was applied to the motor nerve.

Two types of experiments were performed on the gastrocnemius of the decerebrated cat: 1. Variation of initial tension, either by augmentation or by inhibition of the proprioceptor tonus, while maintaining the same length. The final total tension produced was independent of the initial tension. Comparing the results obtained at different initial lengths it was found that the total final tension was a straight line function of the length. 2. Maintenance of the same initial tension under different conditions of proprioceptor tonus, which required varied initial lengths. At the same initial tension the final total tension was again a function of the length.

Further experiments with the gastrocnemius and rectus muscles of the cat, and with the gastrocnemius and sartorius of the frog, showed that up to about the maximum physiological length of the muscle in the body, the final total tension was strictly a straight line function of the length, while the initial tension followed a hyperbolic curve.

About the maximum physiological length of the muscle and beyond, other factors appear whose significance is not quite clear at present.

Oestrual behavior in surviving decorticate cats.[1] PHILIP BARD. Department of Physiology, Johns Hopkins University School of Medicine.

[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

Oestrual behavior in cats represents a specific pattern of response. On entering into "heat" the animal engages in excessive rubbing, frequently emits a curious low vocalization and tends to assume a characteristic crouching posture in which it executes treading movements of the hindlegs. These courtship activities often occur spontaneously but they are brought forth or intensified by the presence of a male. During oestrus treading in a crouch can be induced by gentle mechanical stimulation of the external genitalia and, in many individuals, by grasping the name of the neck or rubbing the head. Following intromission or artificial stimulation of the distal vagina a marked after-reaction occurs. Varying somewhat in different individuals this consists essentially of vigorous or frantic rolling, squirming, rubbing and licking.

A female cat, in which all neocortex had been removed, twice entered spontaneously into oestrus during a survival period of twenty-eight months. At each of these times mechanical stimulation of the vulval region evoked typical crouching and treading, and mating or artificial vaginal stimulation was followed by a definite but somewhat incoördinate after-reaction (rolling and head-rubbing).

After finding that full oestrus with all its attendant behavior can be induced in normal or ovariectomized cats by administration of oestrin three decorticate cats were prepared in an attempt to test further the capacity for sexual display in such preparations. Two had been castrated and the responses of all three to oestrin were determined before the cerebral ablation. The results show conclusively that the feline oestrual behavior pattern is not dependent on the cerebral cortex. Under the influence of oestrin crouching and treading were easily evoked by appropriate cutaneous stimulation and even developed spontaneously. Mating occurred in normal fashion. After-reactions were definite, but they were less well executed and more limited in scope than before decortication. The threshold for oestrin seemed to be raised.

Determination of acetone in the expired air. S. B. Barker (introduced by William H. Chambers). Department of Physiology, Cornell University Medical College, New York City.

It has long been recognized that acetone, the most volatile of the three "acetone bodies," can be excreted through the lungs as well as through the kidneys. However, present methods for the quantitative determination of exhaled acetone are not such that they can be applied directly to respiratory metabolism studies. Since such a combination is especially desirable in the accurate estimation of ketone excretion, a method has been devised for use with the open-circuit type of respiration apparatus. Three Williams bottles, each containing 25 to 30 cc. of a 2 per cent sodium bisulfite solution made up to one liter with distilled water, are introduced into the air current for the duration of the experiment. The absorbed acetone is determined on 50 cc. aliquots by means of the Denigès-Van Slyke mercury chromate precipitation. Using these amounts, one obtains the total acetone in each absorber directly from the weight of precipitate, since 1 mgm. of acetone yields 20 mgm. of precipitate. Proof of the accuracy of the procedure was secured by 99 per cent recovery of acetone added in varying amounts to the absorbing solution as well as by 97 per cent average recovery of acetone volatilized in the respiration chamber used for animal experiments.

The method has been tested under many varied conditions, and results in the study of artificially increased metabolism of depancreatized dogs

have shown that the collection of air acetone is indispensable to an accurate determination of changes in ketone excretion. The resting level of acetone in the breath of one diabetic dog was 46 mgm. per hour with a ketonuria of 262 mgm. Exercise increased the breath acetone to 114 mgm., while the urinary excretion dropped to 238 mgm. After dinitrophenol total acetone was practically unchanged but the air fraction increased presumably with the respiratory ventilation. Following the administration of acetone or acetoacetate to normal dogs, significant amounts were excreted as acetone in the breath.

On the mechanism of the susceptibility to insulin in hypophysectomized dogs. B. O. BARNES and A. S. DIX (by invitation). Department of Physiology, University of Chicago.

Under the laboratory conditions in the present experiments, normal dogs fail to show convulsions after receiving intravenously two units of insulin per kilo body weight. However, after hypophysectomy, convulsions regularly occur with doses of 0.3 to 0.25 unit per kilo. The present experiments were undertaken to see if the hypophysectomized animals required more glucose to maintain a normal blood sugar after receiving insulin. Twelve dogs were used, six of which were normal, five hypophysectomized, and one thyroparathyroidectomized. (No change in the convulsive dose of insulin has been found in a series of thyroparathyroidectomized dogs). The experiments were carried out three weeks to six months after the operations, at which time the animals were all in excellent condition. After a fast of twelve to eighteen hours the dogs were placed in a comfortable hammock, in order to facilitate constant intravenous injections. They were not anesthetized or blindfolded but were kept as normal as possible. After a control blood sample was taken, two units of insulin per kilo body weight was injected intravenously. About ten minutes later glucose administration (30 per cent solution) was started intravenously in the fore leg. The rate of injection was gradually increased during the first twenty minutes after which it remained constant. By trial and error a rate was found which would maintain the blood sugar approximately normal. Blood sugar estimations were made at five minutes intervals during the first part of an experiment and at fifteen to twenty minute intervals thereafter.

In order to prevent large changes in the blood sugar, approximately 25 mgm. of glucose per kilo per minute was necessary after the first twenty minutes. The blood sugar fell below the control when 22 mgm. or less was administrated and rose above the control when 27 mgm. or more was administrated. No difference could be detected in the normal, the hypophysectomized, or the thyroparathyroidectomized dog. The experiments seem to indicate that the hypophysectomized animal does not require more sugar to counteract the action of insulin, but he is less able to mobilize glucose in order to combat the hypoglycemia.

The effect of vagotomy on gastric motility in man. LOUIS E. BARRON (by invitation), GEORGE M. CURTIS and WILLIAM T. HAVERFIELD (by invitation). Department of Medical and Surgical Research, The Ohio State University, Columbus, Ohio.

Gastric motility was investigated in a patient with vagotonia. The baloon and kymograph method was employed. Observations were made

during a preliminary control period, during subsequent atropine medication, and after left abdominal vagotomy. The usual duration of each experiment was five hours. Observations were made in the morning about 14 hours after the preceding evening meal. Thirteen experiments made during the control period revealed intense gastric motility, which frequently persisted throughout the observation period. During preoperative atropine medication nine experiments revealed a slight decrease in the duration of gastric motility associated with a corresponding increase in the duration of quiescence. There was a slight decrease in the number and amplitude of the contractions. The interval between contractions was moderately increased. Following resection of 2.5 cm. of the upper left abdominal vagus nerve twelve observations revealed a marked decrease in the duration of gastric motility associated with a corresponding increase in the duration of quiescence. There was a definite decrease in the number and amplitude of contractions, and a pronounced increase in the interval between contractions.

This patient returned to the University Hospital for reinvestigation approximately six months after the left vagus resection. Seven experiments were made. The results were similar to those of the previous series soon after the abdominal vagotomy.

Fluoroscopic observations on the total emptying time of the stomach were also made. The patient was given a test meal consisting of three ounces of cream of wheat, two ounces of barium sulphate, and sufficient water to form a paste. The average emptying time during the control period was six hours and 49 minutes. During atropine medication, the average emptying time was five hours and 12 minutes, an average difference of one hour and 37 minutes. Following left abdominal vagotomy, the average emptying time was two hours and 50 minutes, approximately four hours sooner than during the control period. Six months after vagotomy, the emptying time of the stomach was two hours.

Racial variation in the crop-gland response of doves and pigeons to prolactin.

ROBERT W. BATES (by invitation), OSCAR RIDDLE and ERNEST L. LAHR (by invitation). Carnegie Institution, Station for Experimental Evolution.

Within races of doves and pigeons of our colony we previously reported a marked genetic difference in the response (weight increase of crop-gland) to a relatively high dosage of prolactin. Subsequently we have obtained data for differences obtainable at a much reduced level of dosage (one-twenty-fifth). When the crop-gland weights at the two dosage levels are converted into physiological (bird) units the relative discrepancy in response (between most and least responsive races of doves) is found to be 20 times on the higher dosage and only 4 times on the lower dosage. This is still a very significant and troublesome difference. But, on the other hand, among our races of common pigeons the wide difference in the (racial) response was essentially maintained at both levels of dosage.

The slope of the line relating dosage and response, as this was previously published, has been confirmed on both doves and pigeons—except that we have found it applicable to the crop-gland weights from the less responsive races of doves only when the gland weights obtained are less than one gram. In this fact lies the explanation for the greater discrepancy in response in doves at the high dosage level.

In view of the observed racial differences comparable assays of prolactin by the crop-gland test can be obtained in different laboratories only by general adoption of *a*, a standard race of dove or pigeon, or *b*,—the more practical method—a standard prolactin preparation with which to standardize or evaluate the response obtainable with the race of pigeons used in any particular laboratory.

Temporal and spatial summation of peripheral impulses with activity of the brain. S. HOWARD BARTLEY. Laboratory of Neurophysiology, Washington University Medical School, St. Louis, Mo.

Cortical responses to paired, triple and trains of rapidly repeated electric shocks to the optic nerve indicate that the activity of any element or group of elements in the brain is rhythmic; that the period of the rhythm is very long ($\frac{1}{5}$ second) as compared to the cycle of events possible in nerve fibres; and that the cortex may respond to frequencies much greater than that represented by the afore mentioned rhythm.

Typically, when two moderate shocks are used, the response to the second will not occur till their separation is at least 80 sigmas. As the separation is made greater, the response to the second grows larger till an interval of about 200 sigmas is reached, after which it again becomes smaller. If three shocks are used, the interval between the second and third to produce a threshold third response depends on the interval between the first two. When shocks are repeated at the rate of about 20 per second, there is a larger response to the first, and to only some of the others, till finally a small response appears to each stimulus. The course of events suggests that the final state of responding to each shock is one in which one group of elements responds to one shock and others to the succeeding ones till the first group has lost its refractoriness and the cycle of events is repeated.

Paired photic stimulation of the retina (each of the two flashes to a different retinal area) follows rules similar in general to the electric shocks to the nerve, when one considers the cortical points recorded from. For each flash, a point can be found which will respond to it and not to the other; likewise an intermediate point can be found which will respond to either or both. The size of the response measured at either one of the first mentioned spots is no greater to both flashes given simultaneously than to one alone. At both the intermediate point and the other two, facilitative and inhibitive effects occur, depending upon the temporal relation of the two flashes.

Further studies of the relation of the degree of reduction of intracellular cytochrome to the oxidation-reduction potential of cell suspensions. J. PERCY BAUMBERGER and ROYCE K. SKOW (by invitation). Department of Physiology, Stanford University, California.

Baumberger, Fahlen, Skow and Bardwell, Proceedings of the Fifteenth International Physiological Congress, Leningrad (1935), reported that the degree of reduction of intracellular cytochrome and the potential of cell suspensions shows close agreement. In the work here reported the time factor was taken very accurately into consideration. The absorption of light of known wave length by a cell suspension and the potential of the cell suspension were graphically recorded on the same film. This photographic record showed that changes in the "oxidation-reduction

potential" of the external milieu, induced by different oxygen pressures, were simultaneous with comparable changes in the degree of reduction or oxidation of cytochrome. Simultaneity of change in these two factors was not apparent with platinum electrodes, but it became evident when the dropping mercury electrode was used. The use of the Heyrovsky dropping mercury electrode as an oxidation-reduction electrode has been reported by Müller and Baumberger (1935). This electrode is preferable to platinum, in following temporal changes in potential, because it comes into rapid equilibrium with the solution while platinum is known to lag in poorly poised systems.

Reduced cytochrome still existed at the highest potential to which the cell suspension could be driven by oxygen. It would therefore appear that cytochrome is the principal coupled oxidation-reduction system of high free energy in yeast cells and is an upper limiting factor of oxidation-reduction intensity.

At potentials where oxidized and reduced cytochrome *C* occur in finite ratios the oxidation-reduction intensity, of that portion of the cell where the cytochrome occurs, is in very close agreement with the potential of the external milieu. From this evidence, it would seem unlikely that differences in oxidation-reduction intensity within the cell could exist except in molecular dimensions.

Observations on the effects of meals on cardiac output estimated by blood pressure changes and by acetylene. H. C. Bazett, J. C. Scott (by invitation), M. E. Maxfield (by invitation) and M. D. Blithe (by invitation). Departments of Physiology, University of Pennsylvania and Hahnemann Hospital, Philadelphia.

The estimation of stroke volume from blood pressures and pulse wave velocities previously reported has been continued; outputs determined by calculation and by acetylene have been compared before and after meals. Estimates have been made 45 to 142 minutes after the meal, when the cardiac output might be expected to be relatively steady. In eleven experiments in the basal state the two methods showed a mean discrepancy of $\pm 13\%$. The basal value by acetylene was 2.07 ± 0.20 (mean deviation), and that by calculation 2.20 ± 0.27. After meals the mean value by acetylene was 2.59 ± 0.19 and by calculation 2.70 ± 0.36. The mean discrepancy between individual comparisons after the meals was 11 per cent. Occasionally big discrepancies were present, but in part these appeared due to the impossibility of making the estimates by the two methods simultaneously. The method of calculation appears to be valid for such conditions.

Pulse rate changes after a large meal were greater than those reported by Grollman or Gladstone; systolic pressure usually rose, but diastolic pressure by no means always fell. With a fast pulse rate a rise in diastolic pressure was not uncommon. Estimation of the peripheral resistance from the relation of mean pressure to cardiac index usually showed a marked fall. On the other hand, pulse wave velocities in the central vessels invariably increased, even when both systolic and diastolic pressures fell.

Changes in stroke volume were inconsistent; commonly there was an early reduction followed by a later increase. Electro-cardiographs were also obtained. Usually increases in stroke volume were accompanied by

a relative increase in the Q-T interval when this was compared with the square root of the cycle, but this was not invariable. Changes in the duration of mechanical systole were more irregular, but they invariably differed from those in the Q-T interval. Before a meal the 2nd sound was observed at, or slightly after, the end of the T wave; after a meal the T wave always outlasted the 2nd sound, usually by as much as 20 to 30 σ.

The effect of orally administered malt amylase on the digestion of starch. J. M. BEAZELL and C. R. SCHMIDT (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School.

The ingestion of malt amylase in conjunction with a cereal test meal appreciably augments the digestion of starch in the stomach. In 8 human subjects the starch digestion in the stomach at the end of 45 minutes averaged 45 per cent when the test meal alone was ingested. When malt amylase was added to the meal the digestion in these same subjects averaged 68 per cent at the end of 45 minutes. The same experiment carried out on dogs, where salivary amylase is of no consequence, yielded even more striking results. In this case the digestion at the end of an hour without malt amylase averaged 10 per cent, whereas with malt amylase it averaged 30 to 50 per cent. Ten dogs were used in the experiment.

Five normal dogs on a diet containing 56 per cent starch spilled negligible quantities of starch in their feces. After separating the pancreas from the duodenum so as to exclude pancreatic juice from the intestine, the feces of these dogs while on this diet averaged 40 to 60 per cent starch. When malt diastase was added to the diet the feces averaged 15 to 20 per cent starch.

These results indicate that malt amylase is of definite value in increasing the digestion and consequently the absorption of starch, especially in the presence of deficiency of ptyalin action in the stomach and amylopsin action in the intestine.

The variability of the energy of metabolism in normal persons. JOSEPH BERKSON and WALTER M. BOOTHBY. Division of Biometry and Medical Statistics, and the Section of Clinical Metabolism, The Mayo Clinic, Rochester, Minn.

The variability of determinations of basal metabolism in male and female human subjects has been studied with regard to the form of distribution (Gaussian) and the quantitative estimation of its value. The variability of repeated determinations of metabolism in the same individual (intra-individual variability) was studied in a series of determinations made in the metabolism laboratories of the Mayo Clinic, and in a series obtained from published data of various authors. The variability of determinations made on different individuals (total inter-individual variability) was studied in relation to mean values given by the Mayo Foundation Normal Standards of Basal Metabolism. From equational relationships the variability of the mean metabolism of different individuals was estimated from the intra-individual and total inter-individual variability.

Changes in heart rate accompanying exposure of the carotid gland region to lowered blood oxygen tensions. THEODORE G. BERNTHAL. Department of Physiology, University of Michigan.

Reflex alterations of heart rate resulting from lowered oxygen tensions of the blood supplying the carotid sinus-carotid gland region were studied.

The carotid sinuses and carotid bodies of dogs were perfused at constant mean pressures with heparinized normal arterial blood. At intervals, known changes in oxygen tension of the perfusing blood were produced and the accompanying alterations of heart rate observed.

In dogs not subjected to artificial pulmonary ventilation, lowering of the oxygen tension of the blood supplying the carotid gland region resulted in some instances in diminished pulse rates. In the majority of cases, however, the effect of low oxygen tension in the carotid gland region was to produce a small increase of pulse rate. This effect was reversed in those instances in which constant artificial pulmonary ventilation was substituted for natural ventilation.

Latent addition in muscle and nerve. H. A. Blair. Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

Using as a representation of the local excitatory process the differential equation,

$$\frac{dp}{dt} = KV - kp$$

where p is the excitatory state, V is the stimulus and K and k are constants, the subsidence of the excitatory state following an inadequate stimulus is given by

$$\frac{dp}{dt} = -k'p$$

the prime being used to allow for the possibility that the subsidence is different with the current off than on. If, for latent addition, two rectangular stimuli of equal but variable strengths, V, and of equal durations, t_1, are separated by a variable interval, t_2, the integration of the equations above gives as the relation for the just adequate values of V to the intervals t_2,

$$\frac{V - R}{V} \times \frac{K + k\alpha}{K} = e^{-kt_1} - e^{-k't_2}\,(e^{-kt_1} - e^{-2kt_1})$$

where R is the rheobase and α is the result of assuming a threshold for p equal to $h - \alpha V$, h and α being constants. This equation is applied easily to latent addition data if at the same time a strength-duration curve is obtained; for the latter, according to the first differential equation, is representable by

$$\log \frac{V}{V - R} = kt + \log \frac{K + k\alpha}{K}$$

and it will therefore supply all the constants but k' of the latent addition relation. It is found for the α excitability of the frog's sartorius muscle and for the sciatic nerve that the subsidence of the inadequate excitatory state is strictly exponential within the probable errors. The constant k'

is about 50 with muscle, and about 5000 with the nerve, at 20°C., so that the excitatory state subsides, respectively, to $1/e = 1/2.72$, approximately, of its initial value in 20 milliseconds and 0.2 millisecond. In both cases, the subsidence is much faster, about 2.3 times in muscle, and 1.7 times in nerve, with the current off than on.

Physiological studies of a condition of abnormal photosensitivity in man. HAROLD F. BLUM and ROBERT J. WEST (by invitation). Division of Physiology of the University of California Medical School, Berkeley.

The opportunity to study a case of *Urticaria solare* has made possible extensive measurements of the photophysiological characteristics of this rare condition, which are of general interest as a demonstration of a peculiar disturbance of metabolism in man. A response which answers the description of the "triple response" of Lewis, occurs within a few minutes following the exposure of the individual to relatively intense blue light. The individual responds normally to erythemic ultra-violet radiation.

We have found that the period of radiation required to produce a minimum erythema of this abnormal type (the threshold time) can be measured with an error of less than 15 per cent. The response obeys the reciprocity law; *intensity* $\times$ *time* = *a constant*, so that the threshold time may be used as a measure of the sensitivity under any condition of constant illumination. Studies over a period of time show fluctuations of the sensitivity, but no trend which would reveal a relationship to diet or other factors of regime.

Studies of the effect of temperature of the threshold time and the rate of appearance of the response indicate that the former has a temperature coefficient of about 1.4, and that the latter has a very high temperature coefficient not measurable at some temperatures. This is best explained by assuming that the threshold time is controlled principally by a photochemical reaction, and that the rate of appearance is controlled principally by a thermal reaction. The behavior of the latter fits very well with that described by Lewis for urticarial responses, and the "triple response" from histamine pricks. Thus it would appear that the response is based on a photochemical reaction which sets off the release of a histamine-like substance in the skin.

Studies of the relative sensitivity to various wave lengths show that only radiations between 3900 A. U. and 5300 A. U. elicit this response. They indicate further that there are two maxima of sensitivity at approximately 4500 A. U. and 4900 A. U. with a minimum at about 4600 A. U. The region of sensitivity corresponds with the region of the absorption spectra of the carotinoid pigments, and the two maxima further suggest that the photochemically active pigment is a carotinoid since these pigments show characteristically two absorption maxima. Carotene would appear as the most likely of the group.

Experiments on the photosensitization of skin by the injection of carotinoid pigments will be discussed.

The effect of sympathin on blood sugar. R. C. BODO and A. E. BENAGLIA (by invitation). The Laboratories of Physiology in the Harvard Medical School and the Department of Pharmacology, New York University College of Medicine.

The experiments which are reported here were carried out on cats. The following operation was previously performed on them: the right adrenal was removed, the left adrenal denervated and demedullated, the liver denervated, the right superior cervical ganglion extirpated.

Sympathin was produced in the following ways: 1, by electrical stimulation of the cardioaccelerator nerves (on both sides simultaneously) and 2, by emotional excitement and struggle.

The experiments, in which electrical stimulation was used, were performed under chloralose anesthesia. Chloralose was selected because it was found not to alter the normal blood sugar level (that remaining uniform throughout the entire experiment), not to interfere with the typical hyperglycaemia caused by adrenalin and not to interfere with the production and manifestations of sympathin (using the denervated nictitating membrane as an indicator). For electrical stimulation faradic currents were used in strengths that did not cause visible contractions of the neighboring muscles. The stimulation lasted each time one minute and was repeated at 5 to 8 minute intervals usually ten times in an experiment. The results were: increase in heart rate, contraction of the denervated nictitating membrane and a rise in blood sugar.

Electrical stimulation of the pectoral muscles causing widespread contractions did not have any effect either on the nictitating membrane or on the blood sugar level.

The second series of experiments were performed without anesthesia; the cats having been frightened with barking dogs or made to struggle on an animal board.

In these experiments also, depending upon the degree and duration of the excitement and struggle respectively, a rise in blood sugar occurred.

From these experiments it is concluded that sympathin, produced either by electrical stimulation of the sympathetic nerves or by emotional excitement and struggle, causes a rise in blood sugar.

A permanent closeable pancreatic fistula,—a description of the operation and some new experimental data. W. N. Boldyreff, N. O. Byland and W. F. Martin (introduced by W. R. Bloor). Battle Creek Sanitarium, Battle Creek, Michigan.

A short history of various methods for making a pancreatic fistula will be given; their peculiarities will be pointed out and the difficulty of conducting experiments on animals with a pancreatic fistula will be reviewed. The pathological changes which sooner or later develop in all such dogs will be briefly listed. Of all the older methods, that of Ivy (similar to that of Minkowski and Hedon) is recommended as the best.

Our procedure for the production of permanent closeable fistula of the main pancreatic duct is essentially as follows: A glass cannula is tied in the usual manner into the pancreatic duct. A rubber tube is attached to this cannula so that its proximal portion is inside the abdomen surrounded by great omentum, and its distal portion lies under the skin. The tip of this rubber tube is cut flush with the skin and is corked. During the experiment the cork is removed and the juice flows outside freely. Some of the dogs so operated lived in our Laboratory for a year or as long as eighteen months in perfect health.

The effect of various substances, particularly that of insulin, on the secretion of the pancreatic juice will be reported.

The sphincter of Oddi in the dog. E. A. BOYDEN and R. A. SCHWEGLER (by invitation). Departments of Anatomy and Obstetrics, University of Minnesota. (Read by title.)

On the basis of wax reconstructions of human embryos and fetuses (see April Supplement, Anat. Rec.) and on the basis of similar reconstructions of dog fetuses, it has been possible to confirm the existence of an intrinsic musculature around the intramural portion of the common bile duct in higher mammals.

In the new-born puppy, the portio duodenalis may be divided into two somewhat overlapping zones. The proximal two-fifths consists of a funnel-shaped passage through the intestinal musculature, which corresponds to the antrum of Westphal. Pending study of early embryonic stages—to establish the original limits of the window through which the duct passes—opinion is reserved as to whether all of this sheath is derived from extrinsic (intestinal) muscle.

The distal three-fifths consists of a series of U-shaped bundles of fibers that surround the bile duct as it traverses the submucosa. These constitute the sphincter choledochus proprius. The open ends of these U-shaped bundles are attached to the circular muscle layer of the gut below the level of the antrum. The closed ends form upward directed loops which extend even into the funnel, where for a short distance they constitute the inner of a double sheath of fibers around the bile duct. The lowest of these loops is thicker and more isolated than the rest and may be called the sub-terminal sphincter. It tends to form a figure 8 with the sphincter of the pancreatic duct. Distal to the figure 8 is a delicate ring-shaped band which encircles both orifices.

These observations confirm the experiments of Lueth '31, who noted that when a cannula was advanced through the portio duodenalis, the pressure fell in steps and the distal zone bulged before the orifice opened, thus implying a terminal sphincter.

Aside from the absence of an ampulla of Vater and a papilla, the intramural segment of the dog differs primarily from the human portio duodenalis in the extent to which it is exposed to compression by intestinal muscle. This suggests a possible explanation for species differences in the rate of evacuation of the gall bladder.

Comparison of the buffering power of saliva obtained under chorda tympani stimulation with that obtained with pilocarpine. CHARLES R. BRASSFIELD and VIVIAN G. BEHRMANN (introduced by Robert Gesell). Department of Physiology, University of Michigan.

Samples of saliva obtained under chorda tympani stimulation from the submaxillary glands of anaesthetized dogs were equilibrated at 37°C. with CO_2 tensions ranging from 0 to 100 mm. Hg. pH determinations were made on these samples by means of the glass electrode and curves of reaction were plotted. These curves were compared with those determined on saliva obtained under pilocarpine stimulation from the same animals.

The initial reaction of the two salivas collected under oil from the same animal were approximately the same with the chorda tympani saliva usually having a slightly higher pH than the pilocarpine saliva.

A comparison of the curves of reaction of the two salivas revealed a marked similarity in their buffering power.

Between CO_2 pressures of 0 and 30 mm. Hg the pH changed considerably

showing a decrease of one pH unit on increasing the CO_2 pressure from 0 to 30 mm. Hg. Between tensions of 30 and 100 the pH curve was less steep. The decrease in this region was 0.4 pH.

A comparison of serum sodium and chloride levels in adrenal insufficiency and various experimental conditions.[1] S. W. BRITTON and H. SILVETTE.[2] Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Studies of adrenal insufficiency within the past two years have led to a rather widespread belief that reductions in serum sodium chloride constitute the serious and specific disturbance after adrenal removal. A number of widely different operative and other experimental procedures have now been found to upset sodium and chloride balance. Almost invariably the change is in the direction of depletion. In several series of experiments, for example in nephrectomy and pancreatectomy, the serum sodium and chloride losses were far greater than in adrenalectomized animals (cats). Simple abdominal operations usually gave rise to considerable decreases in serum sodium and chloride, and intraperitoneal homotransplants of small portions of a number of different tissues (spleen, liver, kidney) resulted in sodium and chloride reductions similar to those which follow adrenal removal. Long-term fasting brought about approximately the same diminutions.

Correlated with the observation that adrenalectomy is followed by *rises* in serum sodium and chloride in some animals (opossum, marmot), the results suggest an indirect and non-critical influence of adrenalectomy on salt balance in the organism.

Glucose distribution between blood plasma and cells in adrenal insufficiency.[1] S. W. BRITTON, H. SILVETTE[2] and R. KLINE (by invitation). Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

The importance of carbohydrate changes in adrenal insufficiency has been shown in earlier papers. An investigation of glucose distribution between blood corpuscles and plasma has recently been carried out. Comparison has also been made with the conditions which are observed in insulin shock and hyperglycemia following pancreatectomy and glucose injection.

In a series of normal cats the ratios were found to be as follows:—For plasma-sugar/blood-sugar, 1.09 — 0.01; for plasma-sugar/cell-sugar, 1.32 ± 0.01. In cats showing symptoms of adrenal insufficiency the respective ratios were 1.17 ± 0.02 and 1.55 ± 0.07. This relative erythrocytoglycopenia appears to be related significantly to carbohydrate deficiencies which have been previously described in adrenalectomized animals.

The hypothalamic control of sympathetic rhythms. D. W. BRONK, F. H. LEWY (by invitation) and M. G. LARRABEE (by invitation). Johnson Foundation, University of Pennsylvania.

[1] Aided by a grant from the Rockefeller Foundation.
[2] E. R. Squibb and Sons Fellow in Physiology.

In a previous communication it has been shown that the impulses in sympathetic nerves, as for instance those from the stellate ganglia of the cat, are frequently grouped into rhythmically recurring waves which are sometimes synchronous with the heart beat or respiration.

In order to investigate the rôle of afferent impulses from the viscera in the determination of this rhythmic activity and in the reflex control of the sympathetic centers we have stimulated alternatively the central end of the carotid sinus, aortic or vagus nerves with stimuli of various frequencies while recording the efferent impulses in a nerve from the stellate ganglion. With frequencies above 30 to 40 a second there is usually an inhibition of the discharge and subsequent escape if the stimulation be prolonged. When the stimulation is stopped there is frequently a second period of inhibition. At lower stimulus frequencies the efferent impulses become grouped into large waves whose frequency corresponds to that of the stimulus. Thus the activity of the sympathetic nerve cells is set into resonance with the afferent impulses.

Because of the large body of evidence which relates the hypothalamus to sympathetic functions we have explored that region for activity related to these afferent and efferent rhythms. By means of concentric needle electrodes inserted into the region of the nucleus hypothalamicus lateralis we have observed potential waves resembling those found in the efferent sympathetic nerves. By electrical stimulation of this region we have been able to initiate corresponding volleys of efferent sympathetic impulses. And by stimulating those afferent nerves which, as stated above, drive the sympathetic motor cells we have produced rhythmic fluctuations of potential in the hypothalamus.

Section of the brain stem anterior to the hypothalamus causes an increased sympathetic discharge. The grouping in the efferent nerves is reduced by section just caudal to the hypothalamus and is abolished by transecting at the level of the acoustic tubercles.

These experiments indicate the importance of the hypothalamus and afferent impulses from the viscera in determining the rhythmic activity of the sympathetic system.

Respiration of local brain regions. Technique and applications. Norris L. Brookens (by invitation), Leon Ectors[1] (by invitation), and R. W. Gerard. Department of Physiology, University of Chicago.

With the Horsley-Clark apparatus, a flat ended hypodermic needle (0.8 mm. inside diam.—18 gauge) can be inserted into a brain at known positions. With a close fitting stylus, extending above into an airtight greased sleeve, it is possible to "suck" into the needle a solid plug of tissue of desired size from a determined position. The reverse maneuvers deposit the brain cylinder (usually up to 5 mm. long, weight 2 to 4 mgm.) into the closed end of a capillary of a Gerard-Hartline micro-respirometer. Each is filled with oxygen, alkali (on filter paper and as recording drop) added, and eight capillaries mounted in the apparatus (37°C.). Steady readings (consistent minute by minute) are obtained within an hour; and, since five minutes suffice for one value, fairly continuous curves can be obtained on a number of samples. Controls have demonstrated adequate oxygen diffusion and CO_2 absorption; and tissue injury is a negligible factor, since even mashing decreases respiration only twenty per cent.

[1] Belgian Fellow of the C. R. B. Educ. Found.

Q_{O_2} values for various portions of white matter ranged from 650 to 1800, for grey from 800 to 2500. Respiration of brain tissue is less when taken from deeply than from lightly anaesthetized animals. Specific regional depression by various anaesthetics and other agents is being examined.

A similar needle technique, without the stereotaxic instrument, can be used for studying the respiration of brain cortex, liver and other organs.

The varieties of vagal sensory fibers which modify the respiratory act. JOHN M. BROOKHART (by invitation), ELLIS H. STEFFENSEN (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan.

Quantitative study of the rate of breathing at normal, deflated, and inflated lung volumes during progressive vagal block revealed a convergence of rates as plotted against block-temperature, beginning approximately at 14°C. and ending near 0°C. When convergence was incomplete the effects of deflation and inflation were small and variable at low temperatures. When inflation accelerated breathing (Head's paradoxical reaction) the effect was in direct proportion to the fall in blood pressure. Such acceleration occurred after vagotomy and was abolished by clamping of the carotid arteries.

Results differed considerably in dogs under light barbital anaesthesia. Lung inflation then produced gross acceleration. In one experiment graded positive pressure produced graded acceleration. Negative pressure produced small but ungraded inhibitions. With slightly heavier anaesthesia graded negative pressures yielded graded acceleration, and graded positive pressures gave graded inhibition until higher pressures were reached, when inhibition suddenly gave way to acceleration.

In one dog, with medium anaesthesia, frequent spontaneous periods of changes in basic rate of breathing occurred. During periods of low basic rate, inflation invariably caused inhibition. During periods of accelerated rate inflation caused further acceleration.

In 10 out of 13 experiments deflation, basal, and inflation rates increased with progressive cold block at about 12°C. to give way to later slowing at lower temperatures.

If the gross acceleration from inflation during light anaesthesia is produced by painful sensory stimulation, as it may well be, combined in some instances with irregular proprio-receptor discharge from excessive stretch of the lungs (Adrian), and if the minor acceleration from inflation during heavier anaesthesia is due to an indirect circulatory effect, then our findings are at variance with those of Hammouda and Wilson. If there are two groups of proprioceptive fibers, one inhibitory and the other acceleratory, blocked out at different temperatures, we have been unable to repeat this differential cold block. Our results suggest that the progressive convergence of rates with graded cold block is produced by progressive reduction in the number of conducting vagal fibers. The acceleration at 12°C. requires further study.

Relation in the rabbit of electrically excitable areas of cortex to placing and hopping reactions. CHANDLER MCC. BROOKS and CLINTON N. WOOLSEY (by invitation). Department of Physiology, the Johns Hopkins University, School of Medicine.

In sixteen rabbits the cerebral cortex was explored with voltage-controlled 60 cycle alternating current under nembutal or ether anesthesia.

Rather sharply delimited areas for contralateral facial, masticatory, neck and foreleg movements were found. Hindleg movements were not always obtained, but in seven animals flexion of the contralateral hindleg was elicited from an area just posterior to the foreleg center.

The placing and hopping reactions of the forelegs in normal rabbits are similar to those of rats, cats (Bard and Brooks, 1932) and dogs (Woolsey, Brain, 1933) but are less well developed. In the hindlegs the reactions to contact of the backs of the feet are poor; a response which is probably proprioceptive is present. Hopping reactions are better in the fore than the hindlegs. All limbs resist being placed in abnormal positions.

Following unilateral ablation of the electrically excitable areas (12 animals) contralateral contact placing reactions were absent. Placing in response to gross displacement of the leg and hopping reactions remained but were definitely deficient. These deficiencies were bilateral after removal of electrically excitable cortex of both hemispheres (8 animals). They were not increased by removal of any other portions of the cortex nor were they produced by destruction of electrically inexcitable areas (16 animals). In four cases all cortex except the excitable area of one side was ablated. The legs opposite this intact cortical remnant showed normal placing and hopping reactions.

These experiments show that in the rabbit, as in the other mammals mentioned, the cortical control of placing and hopping reactions is strictly localized. They indicate that the various forms of these reactions which occur in response to contact are wholly dependent on cerebral cortex while the responses to gross displacement are managed chiefly by subcortical mechanisms. Finally, the fact that hindleg deficiencies follow cortical removals is of interest in connection with the question of cortical control of these extremities in the rabbit.

The assay of urinary oestrin and gonadotropic substances during pregnancy.

J. S. L. Browne and E. M. Venning (by invitation). McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Continuous assay of the excretion of oestrin and of gonadotropic substance of the follicle-stimulating type has been made, using morning specimens of urine, in several cases of pregnancy. Usually two or three day's specimens were united and assayed together. Gonadotropic substance was concentrated by the Katzman-Doisy procedure and oestrin was extracted with butyl alcohol and fractionated between this and 3 per cent sodium carbonate. The test animal was the intact 21 day old rat. A positive vaginal smear was taken as a positive reaction to oestrin, and this together with histological evidence of follicle stimulation, as a positive reaction to gonadotropic substance. The carbonate soluble fraction was assayed directly without hydrolysis. The number of days is reckoned from the first day of the last menstrual period. The results of the oestrin assay in one case were as follows.

The most striking feature of the oestrin excretion was its cyclic nature. These cycles continued throughout the whole of pregnancy. The period of the cycles from peak to peak varied from 24 to 29 days. The menstrual cycle of the patient was 28 days in length before pregnancy. This suggests that certain cyclic processes involved in oestrin excretion continue throughout pregnancy. Labor occurred during a low phase of oestrin excretion on day 280, and was accompanied by a sudden sharp rise in the fraction of oestrin which remained in butyl alcohol.

The excretion of gonadotropic substance was followed in five cases, all showed similar changes. It was usually present at some point between days 9 to 15, reappeared about day 25 and increased gradually up to day 32, the rate on this day varying from 200 to 500 units/1. From then on it rose rapidly, reaching a peak between days 52 to 64, the values ranging from 133,000 to 400,000 units/1. About day 67 the rate decreased sharply to 40,000 units/1. After day 120 it remained between 1500 to 5000 units/1. In one case followed to term, there was no change on the day of labor.

The diffusion constant and molecular weight of inulin. JOSEPH J. BUNIM (introduced by Homer W. Smith). Department of Physiology, New York University College of Medicine.

Preliminary measurements have been made of the molecular weight of inulin by the Barger method, but in view of Berner's assertion that inulin crystallizes from alcohol-water mixtures with the inclusion of small quantities of alcohol which cannot be defecated readily, and because contaminating molecular species from this or other sources would vitiate results obtained by this method, it was abandoned in favor of the Northrup-Anson diffusion method. Using the known diffusion constant of HCl at 25°C., diffusion cells made of sintered glass membranes (4P porosity) were standardized at this temperature, and used to determine the diffusion constant of HCl at 37.0°C. The constant so obtained was used to re-standardize these glass membranes from time to time, and as the basis for the determination of the diffusion constants of substances of interest in renal physiology (urea, creatinine, sucrose, phenol red, inulin, etc.). Inulin and creatinine were allowed to diffuse simultaneously, the diffusion of the latter affording a check against streaming or other sources of error. Inulin from dahlia and chicory, purified by a variety of methods, has been examined. The method affords what is believed to be a trustworthy means of determining the effective molecular weight, and of observing the effects of boiling, standing and the presence of salts and other substances on this value. The results obtained by this direct method indicate a higher molecular weight than has been observed by other investigators using a colligative method.

A study of the electrical potential of the cerebral cortex in relation to anesthesia, consciousness and unconsciousness. W. E. BURGE, G. C. WICKWIRE (by invitation), O. S. ORTH (by invitation), H. W. NEILD (by invitation) and W. P. ELHARDT (by invitation). Department of Physiology, University of Illinois, Urbana, Illinois.

A portion of the cortex of the brain of a dog anesthetized with ether was exposed by removing a piece of the cranium and the meninges. The sciatic nerve was also carefully exposed. One non-polarizable electrode was placed against the cortex of the cerebrum and another against the sciatic nerve. A delicate galvanometer was connected with the electrodes. The cortex of the brain of the deeply etherized dog was found to be electro-positive to the sciatic nerve and the galvanometer indicated a flow of current from the positive brain to the negative sciatic nerve of 2 to 3 micro-amperes. Administration of the ether was then discontinued temporarily and as the dog came from under the anesthetic the cerebral cortex became progressively less electropositive and finally there was a reversal of polarity, the cerebral cortex becoming electronegative to the sciatic nerve and when the dog was practically out from under the anesthetic, the

galvanometer indicated a flow of current of 2 to 3 micro-amperes from the positive sciatic nerve to the negative cerebral cortex. Reanesthization of the dog caused the cortex of the brain to become electropositive again and the sciatic nerve electronegative. As a matter of fact, it was not difficult to produce this reversal in polarity between the cerebral cortex and sciatic nerve over and over again on the same dog.

Unconsciousness produced by bleeding and suffocation, as well as by etherization was found to decrease the negative potential of the cerebral cortex and a reversal in polarity. There seems to be a threshold potential of the cortex below which the animal loses consciousness and above which the animal regains consciousness.

It is known that active tissue is electronegative and inactive tissue electropositive. The electronegative cortex is active and conscious while the electropositive cortex is inactive and unconscious. Hence, it would seem that consciousness or unconsciousness is a matter of electrical potential of the cerebral cortex and this in turn is undoubtedly dependent upon the balance between the loss and gain of electric charges passing to and from the brain over the nerves.

Further study on the mechanism of action of ultra-violet radiation in precipitating protein and producing cataract. W. E. Burge. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

Several crystalline pigs' lenses were placed on an ordinary glass microscopic slide. Two No. 16 platinum wires were laid crosswise of the slide 4 cm. apart among the lenses with one end of each wire projecting from the side of the slide. A quartz plate two millimeters thick was placed on the lenses and by gently pressing on the quartz plate a continuous layer of lens material was formed between the plates and in close contact with the platinum wire electrodes. The preparation was covered with a thick piece of cardboard with a narrow slit cut in it and this was so placed that the slit came over one of the electrodes and the lens material. A delicate galvanometer was connected with the two platinum wire electrodes. A quartz mercury arc burner operating on a 220 volt circuit was suspended over the preparation at a distance of 40 cm. so that the radiation passed through the slit in the cardboard, the quartz plate and on to the platinum wire electrodes and lens material. The temperature was maintained during the irradiation around 35°C. by placing the preparation on a block of ice.

Before the irradiation was begun the lens material around the two platinum electrodes was of practically the same electrical potential. Soon after the irradiation was begun, the galvanometer indicated a flow of current from the non-irradiated lens material to the irradiated. At the end of 2 minutes of irradiation there was a current of 0.025 micro-ampere and after 39 minutes a current of 0.275 micro-ampere from the non-irradiated to the irradiated lens material. The application of a few drops of a weak solution of calcium chloride to the irradiated material immediately abolished the electronegativity and current.

It is known that ultra-violet radiation possesses ionizing power and it is assumed that the radiation produced ionization in the lens material. The abolition of the electronegativity and current by the application of calcium is attributed to the combination of the positive calcium ions with the negative ions in the irradiated lens material.

Observations on the cardia in unanesthetized animals. G. E. BURGET and W. E. ZELLER (by invitation). Department of Physiology, University of Oregon Medical School, Portland, Oregon.

Dogs with chronic esophageal and gastric fistulae were used. While the animal lay quietly on a table a balloon was placed in the cardia by way of the esophageal fistula. This balloon was on a fairly rigid tube such that it could be held in place with the hand. Another balloon recorded simultaneously the movement of the cardiac portion of the stomach. In order to initiate a peristaltic wave in the esophagus a balloon on a small rubber tube was placed in the esophagus for a distance of only a few centimeters. This balloon remained collapsed except when a stimulus was desired at which time it was distended for a few seconds. A peristaltic wave thus initiated was followed by its effects upon the balloons in the cardia and stomach. In the animal with vagi and sympathetics intact a relaxation of the cardia and cardiac portion of the stomach takes place (receptive relaxation). Cutting one vagus did not affect this momentary inhibition. In three dogs section of both vagi above the diaphragm did not result in spasm of the sphincter and did not affect relaxation of the cardia in response to a peristaltic wave of the esophagus. Similarly section of the splanchnics was followed by relaxation of the cardia in a normal manner. In two dogs where both vagi and splanchnics were sectioned this inhibition persisted.

While the tone of the cardia and cardiac portion of the stomach may be influenced by extrinsic nerves, these observations indicate that inhibition here following a peristaltic wave of the esophagus is dependent upon an intrinsic nervous mechanism.

The measurement of vasomotor reactions to temperature changes by means of a bath calorimeter. A. C. BURTON (by invitation) and H. C. BAZETT. Department of Physiology, University of Pennsylvania.

A well stirred bath tub heated by an electrical immersion heater was regulated at a constant temperature. A telechron clock recorded the time the heater was on. Comparison of the heat required with and without the subject indicated the heat lost by the immersed surface of the subject, who was sealed in the bath to the neck by a layer of paraffin wax. Rectal temperatures were recorded electrically and oxygen consumption was measured. Direct calorimetry in periods as short as 10 minutes was possible; bath temperatures and surface temperatures were identical.

Bath temperatures between 30° and 37° showed: 1. The heat loss to the bath is independent of bath temperature when this differs from the rectal by 1° to 4°C. A greater difference entails greater loss and increased metabolism. 2. Adjustment is attained by alterations in the effective thermal conductivity of the tissues, measured by the ratio of heat flow per unit area to the thermal gradient. This index varies by a factor up to 6 times. 3. At temperature differences greater than 4°, an increased conductivity accompanies the increased heat production, and presumably depends on the increased circulation to the muscles. 4. There are indications of a seasonal variation of the minimum thermal conductivity. 5. Comparison of heat loss with heat production gives a direct estimate of the change in average body temperature. When the surface temperature is rapidly altered, the rectal temperature gives no indication of the magnitude or direction of this change. Initial paradoxical changes in rectal tempera-

ture normally result from changes in surface temperature in either direction. Use of the formula combining surface and rectal temperature gives much better estimates of average body temperature. 6. Paradoxical changes in rectal temperature accompanying changes in thermal conductivity may be predicted from a physical model. 7. In view of the changes in rectal temperature the observed vascular responses must be mainly reflex in origin. 8. The method is able to demonstrate brief circulatory changes produced by other means.

The effect of gastrectomy in young dogs on calcification of bone. R. A. BUSSABARGER (by invitation), L. G. LEDERER (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

During the course of our study of the effects of gastrectomy on various species of animals, five of a group of seven puppies were gastrectomized, two being retained as a control. The age at which gastrectomy was performed varied from 103 to 172 days. After recovering from the operation the same stock diet *which included cod liver oil* was fed the five gastrectomized and two control puppies. Blood studies consisting of red blood count, hemoglobin (Newcomer), hematocrit, and Price-Jones counts were made at frequent intervals. Because of the occurrence of two pathologic fractures in puppy no. 1 and because of the subsequent appearance of progressive bone deformities in all the gastrectomized puppies, x-ray, blood chemistry consisting of calcium, phosphorus and phosphatase determinations, and bone ash studies were made.

Our studies showed that after an initial weight retardation that lasted for one month the weight curves of the gastrectomies were comparable to those of the controls. However, with the exception of no. 5 the weights of the gastrectomies became constant at a slightly lower level than the constant weight level of the controls. With the exception of no. 5, the gastrectomized puppies had a mild oligocythemic, hypochromic, normocytic anemia which disappeared spontaneously when their weights became relatively constant 220 days after operation.

All of the gastrectomies showed bowing of the front legs. X-ray pictures showed that the bones of the gastrectomized puppies had slightly larger transverse diameters, markedly wider medullae, and much thinner cortices than the controls. No evidences of rickets were seen.

The serum calcium, phosphorus, and phosphatase of the gastrectomized puppies was found to be normal at the 315th post-operative day. Intravenous calcium curves of the "gastrectomies" were comparable to those of the controls. The results of other studies on calcium metabolism and the composition of the bone of gastrectomized puppies will be reported.

The physiology of an invertebrate smooth muscle (retractor of Thyone bryareus). H. G. DU BUY (introduced by A. Forbes). Department of Physiology, Harvard Medical School.

Five retractor muscles attach the stomach of *Thyone bryareus* to the inner body wall. They are histolcgically smooth muscles. Nothing is known histologically about their innervation, but they are connected with the tentacle ring around the mouth, presumably by an ectoneural nerve ring containing ganglion cells.

These muscles show a twitch-like contraction after direct tactile or

electrical stimulation, and a more sustained contraction after indirect stimulation involving the ganglion cells of the ring.

Their electrograms are complex. At least two components can be distinguished, as in other smooth muscles. On direct or indirect stimulation by single induction shocks, the cathode ray oscillograph shows an initial deviation with a latency of about 25 msec., followed by a slower deviation of some 250 msec. duration. Stronger stimuli elicit rhythmical deviations superposed upon the slow wave. Spontaneous contractions also invariably show these short rhythmical deviations.

Curare blocks the response to indirect stimulation, as in the case of striated muscle. Direct stimulation after curare causes only local contraction under the stimulating electrodes. This indicates that normally the excitation wave is distributed along the whole muscle by way of its nerve supply. The conductile and the contractile mechanisms can be separated.

Adrenaline (10^{-4} in sea-water) causes relaxation of a preëxisting contraction.

Isolated muscles show sustained contractions when immersed in solutions of acetylchone as diluted as 10^{-8} to 10^{-9} in sea-water. No eserine is required for this reaction. These muscles are therefore even more sensitive to acetylcholine than those of the leech. Curare, in doses which block neuromuscular transmission, does not prevent the response to acetylcholine The choline-sensitive system therefore lies in the muscular component of the neuromuscular system, and is responsible for the "viscosoidal tone."

This muscle differs from the crayfish claw in that the latter does not react to acetylcholine, and contracts in response to adrenaline.

Urinary output and the regulation of blood volume and composition in experimental hydremia. D. Bailey Calvin. Department of Biochemistry, School of Medicine, University of Missouri.

In a previous report (1933; Ibid., **105**: 135) evidence was offered to show that following massive intravenous injections of isotonic sodium chloride solutions, the blood volume remained greater than normal for periods of at least four hours after fluid administration. The response was not of the same degree in all cases, however.

In an effort to clarify the reasons for the observed variations, further experiments have been done to determine the effect of urinary output on blood volume equilibration. All experimental conditions were similar to those previously described. It was found that in the immediate regulation of blood volume following massive saline injection (8.14 per cent of body weight) the kidneys played an unimportant rôle. Sixty per cent of the injected fluid and chloride may leave the blood stream before any appreciable increase in urinary output has occurred. Following this initial period (10–20 min.), two general types of kidney response were obtained. In the first type, there was a rapid increase in urinary output. At first the urine voided ran very low in chlorides, but at the end of 75 to 100 minutes urinary chloride concentration had reached approximately 1 per cent and by the end of 125 to 150 minutes the percentage excretion of injected chlorides was equal to or greater than the percentage excretion of injected water. With this type of urinary response, hemoglobin and other whole blood constituents indicated, so far as blood was concerned, a complete volume equilibration or even concentration, although a slight dilution was usually observable in the plasma.

In the second type of kidney response urinary excretion was sluggish. Urinary chloride concentration was never as high as in the injected saline solution (0.95 per cent), and at the end of the four hour experimental period chloride excretion was far behind water excretion. The blood picture showed marked retention of fluid, with relative blood volumes remaining 10 to 15 per cent above normal.

Further experiments are in progress to determine the relative importance of solute and water in controlling these phenomena.

Anterior pituitary extracts and liver fat. J. CAMPBELL (introduced by C. H. Best). School of Hygiene, University of Toronto, Toronto, Canada.

In previous experiments (Best and Campbell) it has been shown that the administration of certain anterior pituitary extracts produce a rapid and extensive deposition of fat in the livers of fasted rats. These studies have been continued and evidence on the fraction of the anterior pituitary extracts which contain the active material will be presented. Prolactin and an active gonadotropic preparation, kindly provided by Dr. Oscar Riddle, exert no influence on liver fat. More recently other species (guinea pigs, mice) have been used. It has been found that the guinea pig is much more sensitive to the active substance or substances which produce an increase in liver fat than is the rat. The effect on liver fat is much more pronounced in the female than in the male guinea pig.

The colloid osmotic pressure, nitrogen content and refractive index of turtle blood serum and intraperitoneal fluid. MILDRED L. CAMPBELL (by invitation) and ABBY H. TURNER. Physiological Laboratory, Mount Holyoke College.

As a part of a study of the possible significance of the colloid osmotic pressure of the blood plasma in the lower vertebrates, determinations have been made of the colloid osmotic pressure, nitrogen content, and refractive index of both blood serum and intraperitoneal fluid of a reptile, the turtle Malacoclemmys geographica, both in the normal state and as affected by varying conditions of temperature and feeding. In the following table are listed the averages of determinations on the sera of animals in the normal fall and early spring condition and figures for individual specimens kept approximately three months under the laboratory conditions specified.

CONDITION	COLLOID OSMOTIC PRESSURE	PROTEIN NITROGEN	PROTEIN CONCENTRATION	REFRACTIVE INDEX
	mm. H_2O	*mgm. per cc.*	*grams per 100 cc.*	
Normal, fall	98*	5.90*	3.68*	1.34377*
Room temperature (19–20°C.), feeding	99	6.60	4.12	1.34399
Room temperature (19–20°C.), starvation	39	6.34	3.96	1.34342
Winter temperature (7–10°C.), feeding	72	6.04	3.78	1.34339
Winter temperature (7–10°C.), starvation	66	5.56	3.47	1.34548
Normal, spring	58†	4.46†	2.78†	1.34222†

* Average of five specimens.
† Average of two specimens.

In each group of experimental animals, except those kept at room temperature and fed, there was observed a progressive fall in the colloid

osmotic pressure during the three months in which determinations were made.

The relations of protein nitrogen and refractive indices to the serum colloid osmotic pressure will be discussed.

The colloid osmotic pressure, protein nitrogen content and refractive index of samples of intraperitoneal fluid were consistently lower than those of the corresponding sera, although differences between these two sets of figures were not constant.

Despite the facts that the serum colloid osmotic pressure in some specimens had fallen to half the normal value and that in several cases the colloid osmotic pressures of serum and intraperitoneal fluid were very close, no indications of edema were found in any animal.

Sensitization of denervated structures. W. B. CANNON and A. ROSENBLUETH. Department of Physiology, Harvard Medical School, Boston.

Sympathetic denervation sensitizes smooth muscle to adrenine (Meltzer and Auer, Am. J. Physiol., **11:** 18, 1904). Parasympathetic denervation sensitizes smooth muscle to acetylcholine, as shown by effects on the circular fibers of the iris after removal of the ciliary ganglion (Shen and Cannon, Chinese J. Physiol., 1936, in press). The neurones with cell bodies in the superior cervical ganglion may be regarded as innervated by preganglionic fibers. There is evidence that in the transfer of impulses from preganglionic fibers to the neurones of the ganglion acetylcholine is involved (Feldberg and Gaddum, J. Physiol., **81:** 305, 1934). Does denervation of the ganglionic neurones render them likewise more sensitive? Experiments prove that these neurones are sensitized to appropriate doses of acetylcholine injected intravenously into eserinized animals, as revealed by contraction of the nictitating membrane on the denervated side and not on the normal side, and by disappearance of the phenomenon when, after both ganglia have been removed, the same dose of acetylcholine is repeated. It is also proved by applying adequate solutions of acetylcholine directly to the two superior cervical ganglia and finding that the nictitating membrane then contracts on the denervated but not on the normal side.

Blood studies in acute experimental hyperparathyroidism. A. CANTAROW, J. T. BRUNDAGE (by invitation) and E. L. HOUSEL (by invitation). Laboratory of Biochemistry, Jefferson Hospital and Department of Pharmacology, Jefferson Medical College, Philadelphia.

A state of acute hyperparathyroidism was produced in 6 adult dogs by the injection of 2700 to 3500 units of parathyroid extract[1] (Parke, Davis and Co.), given in frequently repeated doses of 100 to 300 units over periods of 3 to 4 days. The following determinations were made once or twice daily: hematocrit value, blood nonprotein nitrogen, total plasma protein, albumin, globulin, inorganic phosphorus, calcium, diffusible calcium and phosphorus, phosphatase activity and corpuscular ester phosphorus. The plasma proteins were moderately lowered in a few instances by plasmapheresis and by protein restriction. All animals presented the characteristic clinical manifestations of acute hyperparathyroidism, with terminal rise in nonprotein nitrogen and fall in plasma CO_2 capacity.

[1] Parathyroid extract was generously supplied by Dr. E. A. Sharp, Parke, Davis and Co.

Variations in the plasma protein concentrations appeared to have no significant effect upon the degree of elevation of total or diffusible calcium. The inorganic phosphorus showed a preliminary fall in some but not in all cases, and invariably increased with the development of renal insufficiency. Changes in diffusible phosphorus were relatively insignificant. No consistent significant alteration was noted in the ratio of diffusible to total calcium, and this ratio did not change significantly with changes in the total plasma calcium concentration. The plasma phosphatase values showed minor variations, due perhaps to anemia and nutritional disturbances but, in general, increased slightly but more or less steadily throughout the course of the experiment. The corpuscular ester phosphorus values fell markedly, consistently and promptly and, with minor variations, tended to rise somewhat coincidently with the development of renal insufficiency.

It is believed that this striking diminution in corpuscular ester phosphorus, despite repeated bleeding, which tends to produce an increase, is of significance. In view of the reported observations of an increase in this factor following the administration of vitamin D, these findings may be regarded as evidence that the modes of action of vitamin D and the parathyroid hormone are not identical.

A study of population growth and survival in cultures of Paramecium caudatum subjected to mechanical vibration. LESLIE A. CHAMBERS (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania.

Continuous low intensity mechanical vibration of cultures of *Paramecium caudatum* results in complete cessation of reproduction and in a decrease in population density, the rate of which depends upon the intensity of the vibration. It is interesting that such biological action occurs at intensities too low to cause cavitation in water, since no chemical action has been observed in absence of cavitation and since the physiological effects of vibration usually have been attributed to the formation or collapse of such cavities.

While reproduction does not occur during the period of vibration it is almost immediately resumed upon cessation of vibration and proceeds at about the same rate as before vibration except where the time of exposure has been very great or the intensity great enough to destroy a large proportion of the population. In the latter cases there is a brief recovery period before resumption of normal reproductive activity.

At 1000 cycles per second the number of survivors in a culture at time, t, and vibration intensity, I, may be expressed

$$N = N_o e^{-\frac{t}{a - bI}}$$

as a first approximation, assuming that I is a linear function of the electrical input in the experimental apparatus.

Changes in the metabolism of protein and carbohydrate during prolonged fasting. WILLIAM H. CHAMBERS, J. P. CHANDLER (by invitation) and S. B. BARKER (by invitation). Department of Physiology, Cornell University Medical College, New York City.

In earlier experiments on dogs fasted for 20 to 30 days the respiratory

metabolism after ingestion of gelatin indicated that there was a temporary loss of ability to oxidize glucose derived from protein as well as ingested glucose. This has been confirmed recently in 5 experiments with meat protein. After a meal of 500 grams of meat there was a rise in blood sugar, a marked increase in ketonuria, and no significant change from the basal R. Q. of 0.72.

When a longer fast has depleted the body fat the dog (Dann, 1932) or the rat (Benedict and Fox, 1934) may obtain all of its energy from the metabolism of body protein with a quotient of 0.80. The following observations on the metabolism of protein and glucose have been made in 2 dogs in this condition. Between the 30th and 45th days of fast the basal quotients rose from 0.73 to 0.80, while the nitrogen excretion increased gradually from about 100 to about 300 mgm. per hour. The excretion of ketones diminished slowly and disappeared when the urinary nitrogen reached about 200 mgm. per hour and the basal R. Q. was 0.78. On the 47th and 48th days 500 grams of meat were metabolized in the normal manner according to quotients of 0.78 and 0.81, although ketone bodies reappeared in the urine. Two days later the basal quotient had returned to the early fasting level of 0.72 and after glucose ingestion rose to 0.83. In the other dog 50 grams of glucose on the 50th day of fast resulted in a definite elevation of the R. Q. to 0.92. Although some glycosuria was found, a partial restoration of the glucose oxidizing function was indicated. With an exclusive fat diet, 285 grams in 2 days, ketonuria occurred again and a definite protein-sparing action of the fat was exhibited by a fall in urinary nitrogen from 321 down to 87 mgm. per hour. The lower basal R. Q. of 0.75 was unchanged by the ingestion of glucose. Thus with respect to carbohydrate oxidation the condition was similar to the early fasting state.

A slip cover nephelometer adaptation for the determination of fluorescent substances (urobilin). B. B. Clark (by invitation) and R. B. Gibson. Laboratory of Pathological Chemistry, University Hospitals, The State University of Iowa. (Demonstration.)

Light from a nephelometer lamp (B. & L.) with a photoflood light bulb passes through two slits (10 x 25 mm.) in the slip cover in front of the colorimeter cups which are of glass. The slits may be made greater or less vertically by slides operated by rack and pinion and are provided with mm. scales and verniers. The plungers of the colorimeter are removed and the cups filled with standard and unknown solutions. The usual colorimetric procedure is then followed. Observed results when varying dilutions of urobilin and of acriflavine are read against standards are in agreement with calculated values.

The effect of ultraviolet radiation on lens protein in the presence of salts and sugars. Janet Howell Clark. School of Hygiene, Johns Hopkins University.

A previous investigation has shown that radiated lens proteins are precipitated by dilute calcium chloride at the pH of the lens but not by potassium and sodium chloride.

As diabetic cataract has been attributed to the presence of sugar in the lens, sugar was added to extracts of lens proteins in Ringer solution and in 0.5 per cent potassium chloride. Extracts in Ringer solution developed

a slight opalescence on radiation, owing to the small amount of calcium present, and this was not affected significantly by the addition of sugar. However, in the potassium chloride extracts, the addition of 0.1 to 0.2 per cent destrose or levulose resulted in a slight but definite opalescence after radiation. It is possible therefore that the presence of sugar in the lens may result in the precipitation of light-denatured protein.

As it has been stated by Burge that silicates precipitate radiated proteins, sodium silicate was added to extracts of lens proteins in sodium, potassium and calcium chloride. Sodium silicate did not produce any opalescence in the sodium and potassium chloride extracts and prevented the development of opalescence in solutions containing calcium chloride, on radiation. Sodium silicate therefore does not precipitate radiated lens protein itself and prevents the precipitation of radiated proteins by calcium.

Dark adaptation in the insect, Dineutes assimilis. LEONARD B. CLARK (introduced by Selig Hecht). Columbia University and Union College.

The course of dark adaptation was followed in the aquatic beetle, Dineutes assimilis by finding the threshold intensity to which specimens would react after various measured periods in the dark.

Observations were made after light adaptation to intensities of 6.5, 91.6 and 6100 foot candles. The threshold gradually decreased with stay in the dark until a minimum was reached after which it remained constant. With increase in the intensity of the adapting light, the initial threshold became higher, the threshold dropped faster and did not reach the minimum until after a longer time in the dark.

The results are described by an equation similar to that of a bimolecular isotherm. The average values of the velocity constant were 1.32, 1.48 and 1.28 for the three series of data. The average for all readings was 1.33.

The logarithm of the computed instantaneous threshold was proportional to the logarithm of the adapting light over the range of intensities studied.

The excretion of inulin and creatinine in M. rhesus. ROBERT W. CLARKE (by invitation) and HOMER W. SMITH. Department of Physiology, New York University College of Medicine.

Simultaneous clearances of creatinine and chicory inulin in the male and female monkey (amytal anaesthesia) are identical within the limits of analytical error, indicating that there is no tubular secretion of creatinine in this animal. Further experiments are in progress on other primates. The monkey resembles in this respect the dog, sheep, rabbit and seal, observations from this laboratory having shown that in these animals the creatinine/inulin clearance ratio = 1.00.

The urea clearance is relatively lower in the monkey than in dog or man, but shows a similar variation with urine flow.

Electric impedance of Asterias and Arbacia eggs. KENNETH S. COLE and ROBERT H. COLE (by invitation). Department of Physiology, Columbia University and Biological Laboratory, Cold Spring Harbor.

The alternating current resistance and capacity of suspensions of Asterias and Arbacia eggs have been measured at frequencies from one thousand to sixteen million cycles per second. On the basis of a generalization of the Maxwell equation for the resistance of a suspension of spheres, it is found 1, that the plasma membrane capacity is 1.1 $\mu f/cm^2$ for the

Asterias and 0.73 $\mu f/cm^2$ for the Arbacia eggs; 2, that the egg interiors are not electrically homogeneous, about 5 per cent of it being membrane covered material, while the balance has a specific resistance 6 to 8 times that of sea water; 3, that the fertilization membrane of the Arbacia egg has a capacity of about 3.1 $\mu f/cm^2$, with a specific resistance of the perivitelline space about equal to that of sea water.

Effect of testosterone on the mammary gland. J. B. COLLIP, C. S. MCEUEN (by invitation) and H. SELYE. McGill University, Montreal.

Two milligrams of testosterone benzoate in corn oil were injected subcutaneously for 22 consecutive days into 4 intact and 4 castrate 23 day old female rats. In both groups the mammary glands showed acinus development and dense milk was present in the ducts

The action of acetylcholine in the arrest of experimentally induced convulsions in the cat. HELEN C. COOMBS and OTIS M. COPE. Department of Physiology and Biochemistry, New York Homeopathic Medical College, New York.

In this series of experiments (25 cats), the object was to determine the possible effects of sub-lethal dosages of acetylcholine upon experimental epileptiform convulsions induced by camphor monobromide. The technique for the use of camphor monobromide[1] as a standardized convulsant agent has been previously described. (Wortis, Coombs and Pike, Arch. Neurol. and Psychiat., 1931, **26**: 156–161.)

Procedure. Ten to fifteen minutes after establishing the minimal conclusive dose of camphor monobromide for an animal, acetylcholine in varying dosages was injected intravenously 1, within ten minutes before; 2, just before, or 3, during injection of the same dosage of camphor which had been found previously to be the minimal convulsive dose.

Results. It was found that an average dose of from 0.007 to 0.01 cc. of acetylcholine per kilo body weight of the cat, injected either with the camphor, or within ten minutes before its administration, will arrest the clonic convulsion. Restlessness, struggles, deep gasping or rapid respiration, and tonic movements of extention, persist. If the acetylcholine is injected during a convulsion, it does not arrest the spasm, nor does it check the usual persistent after-twitches. When both drugs are used together, death ensues at a much lower dosage of each, than the average lethal dose when administered separately.

	SEPARATELY	TOGETHER
Average minimal lethal dose camphor monobr. cc./kgm, weight	0.109	0.058
Average minimal lethal dose acetylcholine cc./kgm., weight	0.05–0.09	0.009–0.015

This is in line with previous work in which Coombs and Pike (This journal, 1932, **99**: 521) showed that in combined effects of cerebral anaemia plus camphor monobromide or absinthe, the effects of smaller amounts of

[1] The standard solution of camphor monobromide used contains 10 grams of camphor monobromide in 100 cc. 95 per cent alcohol. The acetyl choline used is that of Lematte and Boinot in which 1 cc. contains 0.1 gram of acetylcholine chloride.

the two agents together were proportionally more severe than greater amounts of a single agency. That the combined effects of these two chemical agencies are so potent a summation of the effects of each taken separately emphasizes the fact that they must act upon the same nervous structure. It may also be suggested, that while undoubtedly effective as an arrestor of clonic convulsions experimentally, the use of acetylcholine clinically in cases of epilepsy might be open to considerable further investigation before it is accepted. Moreover, since the primary effects of acetylcholine are said to be over within forty-five seconds to a minute after jugular injection, the question must be raised as to the nature of the substance which is so effective in stopping clonic convulsions from one to ten minutes after injection. Further studies are in progress.

Protein and calcium changes in serum during sleep and rest without sleep. N. R. COOPERMAN (introduced by N. Kleitman). University of Chicago.

It has been repeatedly reported and various conclusions drawn therefrom, that during sleep there is a fall in total serum calcium. This work was undertaken to determine how much of this change occurred in ionizable calcium.

In ten subjects on eleven experimental nights it was found that after five to seven hours of sleep (blood drawn on going to bed and immediately upon awakening) there was a slight decrease in total calcium (average three per cent) and a larger decrease in serum proteins (average ten per cent), resulting in a small increase in ionizable calcium (average five per cent) as determined by the McLean-Hastings nomogram method. In samples of blood drawn after awakening the subject one and one-half to two hours following the onset of sleep there was found a more marked drop in total calcium (average five and one-half per cent), no greater decrease in serum proteins than at the end of the longer periods of sleep, and no change in ionizable calcium. Similar results were obtained on the same subjects when they lay in bed awake for an equal length of time.

In five dogs and a total of fourteen experiments, one to two hours of rest following a short period of exercise (thirty to sixty minutes) resulted in a drop of total serum calcium and serum proteins; the ionizable calcium remaining unchanged. In seven experiments on four dogs a rest period of five to six hours caused a decrease in total calcium and serum proteins and the ionizable calcium rose about four per cent.

It thus appears that whereas there may be a decrease in total serum calcium during sleep and rest, the concentration of the physiologically significant ionizable calcium is unchanged after short periods and consistently increased after long periods of sleep and rest.

Electrocardiograms of the effects of acetylcholine after division of the vagi. OTIS M. COPE and HELEN C. COOMBS. Department of Physiology and Biochemistry, New York Homeopathic Medical College, New York.

In an earlier paper (Cope and Coombs, Proc. Soc. Exper. Biol. and Med., **33:** 480, 1935) we showed that in the intact animal, under nembutal, acetylcholine, in moderate dosages, has a stronger effect upon the QRS complexes that upon the P-waves. It was desired to know what effect, if any, division of the vagi would have upon the electrocardiogram with subsequent doses of acetylcholine.

Procedure. Light ether anesthesia—sufficient to keep the animal from

moving—was used. Control records were taken, and the vagi were cut during the taking of a record. Subsequent records of five seconds each were taken once a minute for five minutes. Acetylcholine—0.1 cc. per kilo was then injected through the jugular vein. It should be remarked that this is a fairly high dosage—just on the borderline of lethal. Electrocardiograms were then taken for one minute, and for at least five seconds of each succeeding minute for at least fifteen minutes, often for half an hour. The results are tabulated below.

CAT NUMBER	HEART RATE		AFTER ACETYLCHOLINE	
	Before vagotomy	After vagotomy	P-waves	QRS complexes
55	176	176	Persist, rate slows to 136	Disappear, reappear gradually, irregularly, rate 70
56	214	250	Disappear	Slow, stop for 5 secs., gradual return to rate 84
57	214	250	Disappear	Slow to 100
58	176	231–270	Disappear	Continue at 100, rise to 270, dropping every 7th beat
59	232	232	Persist	Disappear for 5 seconds, return at 101 for 4 seconds, continue 78
60	250	250	Persist	120, stops, returns to 83
61	186	250	Disappear	Starts at 95, stops for 12 secs., returns to 85
62	210	210	Persist	Disappear, reappear gradually to 84
63	192	236	Disappear	Slow up to 108
64	180	240	Disappear	Slow up to 110

From these preliminary experiments, it appears that in cases where division of the vagi does not change the heart rate, the addition of acetylcholine has a different effect upon the P-waves and QRS complexes than when double vagotomy alters the rate. In the latter case, where the vagi obviously exert a strong effect, not only is heart block produced, but the sino-auricular node must also be affected, whereas, when the vagi play a less marked rôle, the P-waves persist. It should, however, be borne in mind that with such heavy dosages of acetylcholine there is a tendency for the P-waves to disappear in unvagotomized animals. The persistence of the P-waves in some cats argues again for quantitative variations in the distribution of "cholinergic" fibers in the vagus.

Further observations on electro-cauterization of the fetal rat brain. E. L. Corey. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Rats have been operated on *in utero* under procedures standardized to allow comparison with litter-controls as well as different litters. Most of the animals were operated on at the beginning of the last quarter of the gestation period. It was shown that apparently normal survival of fetuses to term may follow destruction of 50 to 75 per cent of the cerebral tissues. In some cases the areas destroyed extended well below the level of the pons.

Abnormalities of development, mostly confined to the head region, are attributable to mechanical factors caused by cauterization. Muscular, respiratory, vocal and other responses of the decerebrate rat fetus are not significantly different from those observed in litter-mate controls. Growth

(weight and length) was also not affected to any noteworthy degree even in deeply cerebral-cauterized embryos.

The effect of dextrose on hepatic gluconeogenesis. LATHAN A. CRANDALL, JR. and IAN S. CHERRY (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Portal, hepatic, and systemic (saphenous or cephalic) venous and arterial blood samples were withdrawn from dogs under Pentobarbital Sodium anesthesia or with London cannulae on the portal and hepatic veins. Arterial blood was obtained from the carotid after exteriorization in a loop of skin or directly from the femoral. Blood sugar (zinc or copper-tungstate filtrates) and lactic acid were determined on these blood samples before and after the oral (London cannula dogs) or intravenous (Pentobarbitalized animals) administration of dextrose.

Our results indicate that after dextrose administration the liver removes this sugar from the inflowing blood; previous reports on this point have been discordant. Although the liver in the fasting animal has been removing lactic acid (and adding dextrose), the removal commonly ceases as the blood sugar rises and lactic acid may even be added to the blood by the liver, although the blood lactic acid may be near the upper limits of normal for the resting animal.

The change from an addition of sugar to the blood by the resting dog to removal of sugar after dextrose, together with the addition of lactic acid to the blood under these conditions gives direct evidence for inhibition of gluconeogenesis after the enteral or parenteral introduction of dextrose, and is not in agreement with the concept that glycogen cannot be formed directly from dextrose. Previous investigators who report that the liver continues to add sugar to the blood even after dextrose administration employed methods of anesthesia which may have disturbed normal carbohydrate metabolism in the liver.

Parathyroid-extract and gastric emptying time.[1] GEORGE CRISLER. Mayo Foundation, Rochester, Minn.

In preliminary experiments on 8 dogs, parathyroid-extract (Lilly) given hypodermically was found to cause a decrease in the emptying time of the stomach on some occasions but an increase on others. A correlation between the change in emptying time and the height of the rise in blood calcium seemed to be the logical first step in attacking the mechanisms involved. It was found in subsequent experiments that a rise in blood calcium up to an increase of about 50 per cent was associated in all of the dogs with a decreased emptying time and that an increase of more than 50 per cent was associated with an increased emptying time. No direct correlation could be made between the size of the dose of parathormone and the changes in the emptying time, nor between the size of the dose, the size of the dog and the emptying time. The relationship at the present time seems to be between the blood calcium level and the length of the emptying time. Other factors are still under investigation and an attempt will be made later to correlate these data with the good results obtained clinically by some from the use of parathyroid extracts in certain cases of gastric pathology.

[1] Work done in Department of Physiology, University of West Virginia.

A technic for the complete fistulization of the gut at any level of the gastro-intestinal tract. B. F. CROCKER (by invitation) and J. MARKOWITZ. University of Toronto.

The ordinary Pavlov fistula or the Mann-Bollman fistula permits sampling intestinal contents but does not admit of the quantitative estimation of the volume of secretion, etc. For this purpose it is necessary to divert all the content to the exterior, where appropriate measurements can be undertaken, and following which the material can be reintroduced. To achieve this a double barrelled Pavlov fistula is made at adjacent points of the gut which is sectioned between the cannulae and each end inverted by oversewing a clamp. The exteriorized cannulae are connected by rubber and glass tubing. With proper care such animals make excellent preparations enabling many varieties of study to be undertaken. The operative mortality is slight.

The respiratory rôle of visceral afferent impulses which travel through the stellate ganglia and the vagi. STUART P. CROMER (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

In a series of ten experiments, (dogs) using 45 seconds of tracheal obstruction as a respiratory stimulus, the following effects in intact, stellecmatized, vagotomized and in animals which had both the stellates and vagi cut, it was found: 1, that when the vagus nerves were cut (stellates intact) the ability of the animal to modify amplitude was only slightly altered; 2, that when the stellate ganglia were removed (vagi intact) the animal retained the ability to alter respiratory rate after a stimulus, although the ability to increase the amplitude of respiration was markedly diminished; 3, that when the stellates and the vagi had been cut, both the respiratory rate and amplitude remained almost unchanged after the stimulus. From this we conclude the impulses controlling respiratory rate, travel mainly in the vagus nerves, while the impulses controlling amplitude travel mainly through those nerves which pass through the stellate ganglia.

Acoustic value of the components of the auditory system in cats. ELMER CULLER (introduced by F. R. Steggerda). University of Illinois, Urbana.

The threshold of hearing in five cats at 125, 1000 and 8000 cycles was first established; some part of the acoustic system was then surgically ablated and hearing again measured; a second and finally a third surgical entry eliminated other components of the system from functional participation, whereupon residual acuity was in each case again tested. By these means the following results were obtained.

1. Destruction of one cochlea, in a cat otherwise intact, is followed by a hearing-loss of 3 to 4 decibels. Destruction of both cochleae eventuates in total deafness to air-borne sounds (no response to tones 125 decibels above the animal's normal threshold).

2. Ablation of a single hemi-cortex is followed by a hearing-loss of the same magnitude—3 to 5 decibels. Statistical analysis indicates that the left hemisphere may be slightly superior in acoustic value to the right; but the disparity, if real, is inconsiderable.

3. In cats with one hemi-cortex ablated, destruction of the hetero-lateral

cochlea causes an additional drop in acuity, owing to exclusion of the *crossed* fibers of the lateral lemniscus, of 15 decibels.

4. In cats with one hemi-cortex ablated, destruction of the homo-lateral cochlea causes an additional drop in acuity, owing to exclusion of the *uncrossed* fibers of the lateral lemniscus, of 13 to 14 decibels.

5. It follows that the uncrossed fibers of the auditory system are virtually equal in acoustic significance to the crossed components. Statistical analysis fails to reveal even a presumptive superiority of the crossed pathways.

6. The evidence discloses a satisfactory safety-factor in the acoustic system, which compares favorably with that found in other physiological systems and with good engineering practise.

7. The above propositions confirm, and considerably extend, conclusions previously derived in this laboratory from dogs.

Dr. Edward Girden, W. J. Brogden and Dr. Fred A. Mettler participated in this investigation.

Motor conditioning in dogs. ELMER CULLER (introduced by F. R. Steggerda). University of Illinois, Urbana. (Motion picture demonstration.)

This film is designed to show the methods by which, in this laboratory, quantitative tests of sensory function in animals are made with a reliability and consistency fully equal to tests on graduate students.

A biologic assay of "international standard" estrin and of some commercial estrin preparations. F. E. D'AMOUR and R. G. GUSTAVSON. Departments of Zoology and Chemistry, University of Denver.

Under the direction of the Permanent Commission on Biological Standardization of the League of Nations Health Organization an international standard for estrogenic substances was established in 1932. The standard material represents pooled contributions from a number of workers and the "International Unit" was designated as 0.1 gamma of this material.

It seemed desirable to subject this material to a thorough biologic assay. Five methods were used, as indicated below. Usually four dosages were given, fifty rats being used for each dose. The results were plotted and the rat unit (the amount necessary to bring 50 per cent of the animals into estrus) determined. The results follow:

SOLVENT	METHOD OF INJECTION	RAT UNIT	
		Gammas	International units
Oil	Single injection	1.3	13
Oil	Multiple injection	1.5	15
Aqueous	Single injection	3.8	38
Aqueous	Multiple injection	0.74	7.4
Emulsion	Multiple injection	0.76	7.6

The commercial preparation indicated below were purchased across the counter. These materials were assayed by the single injection method, 25 rats being used for each dose. The results follow. (By "rat unit" is

meant the number of "International units," according to the labelled content of the ampoule, necessary to bring 50 per cent of the animals into estrus).

MATERIAL	SOLVENT	RAT UNIT
Amniotin	Oil	15
Theelin	Oil	11
Progynon	Oil	8.6
Theelin	Aqueous	240
Progynon	Aqueous	260

Interpretation of the electrogram of certain smooth muscles. H. DAVIS, A. ROSENBLUETH and B. REMPEL (by invitation). Department of Physiology, Harvard Medical School.

The complexity of smooth-muscle electrograms is due in part to the fact that the active cells are small and remote from the electrodes. In the pilomotors of the cat's tail, the advancing wave of activity contributes a spurious leading phase.

Repetitive stimulation of pre- and postganglionic fibers to the nictitating membrane or pilomotors gives evidence of a fundamental independence of the components I and II of the electrogram. At certain frequencies I apparently shrinks, while II grows. This might be due to a shift of latency of II. The latency of I is constant. It might be due to a differential growth of II, overlapping and cancelling I. Either alternative implies independence. Adrenine sometimes has a similar differential effect on I and II. Furthermore, at the beginning of repetitive stimulation, several I potentials may be seen superimposed on a slow summated II wave. II shows a single summated wave in response to a brief rapid series of impulses. The I waves do not overlap or summate, but shrink rapidly to extinction at such frequencies. If the stimulus interval is so brief that the I waves after the first would begin before the subsidence of the first, no corresponding I waves can be detected, and the first response is unmodified.

At frequencies of about 10 per second, far below the optimal for contraction (20 to 25 per sec.), I shrinks and becomes imperceptible within 1 or 2 seconds. This is in clear contrast to skeletal muscle, whose spike potentials are well developed at its optimal frequency.

These data suggest that I and II are distinct phenomena and that neither one is analogous to the spike potential of skeletal muscle.

A convenient method for the determination of the approximate cardiac output in man. JOHN S. DONAL, JR. (introduced by Isaac Starr, Jr.). School of Medicine, University of Pennsylvania.

The purpose of this investigation was to design a cardiac output method more simple than any now in use and to test its accuracy by comparison with more complicated procedures.

Previous investigators have estimated the oxygen content of the venous blood in man by attempting to secure complete equilibrium between the blood and lung air. The unavoidable dilution of any rebreathed mixture by the residual air makes this process difficult and uncertain and the numerous analyses involved are time consuming. A method has therefore

been developed in which approximate equilibrium with the venous blood is attained in a single rebreathing of a measured quantity of nitrogen. Two accurately timed samples taken after mixing is complete and before recirculation begins permit the calculation of the cardiac output from the formula:

$$\text{Cardiac output} = (A - B)/(C - D)$$

where A is the oxygen consumption in cc. per minute, measured in the usual manner; B is the oxygen consumption in cc. per minute during the rebreathing period between the collection of samples; C is the normal arterial oxygen content, or 96 per cent of the blood oxygen capacity, as estimated from a hemoglobin determination; and D is the oxygen content of arterial blood as calculated from the average of the tensions of oxygen and carbon dioxide in the two alveolar samples and a standard nomogram.

The oxygen consumption, B, found during the rebreathing of nitrogen, is calculated from an assumed volume of residual air. It should also be corrected for the unknown change in circulatory rate resulting from rebreathing. B is small compared to A, however, and it has been found that these errors do not greatly affect the calculated cardiac output.

Estimations of basal cardiac output by this procedure have been found to be in good agreement with determinations made on the same subjects by the ethyl iodide method of Starr and Gamble and by the acetylene method of Grollman.

Studies on aortic velocity and the arterial pulse. PHILIP DOW (by invitation) and W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta, Georgia.

The use of a previously described high-frequency optical manometer with hypodermic needle cannulae has made it possible to obtain arterial pressure records of a high degree of reliability. A double cannula has been constructed with which simultaneous upstream and downstream pressures can be recorded, and by calibration these differential pressures have been interpreted in terms of velocity.

Simultaneous records have been made with the double cannula in the aortic arch and a needle cannula in the left ventricle of dogs, for which the only necessary surgery was the exposure of a carotid artery under morphine with procaine locally.

In similar intact dogs a series of simultaneous records have been obtained from the arch of the aorta and successive points at increasing distances down the descending aorta and out the iliac and femoral arteries, demonstrating the development of the peripheral from the central pulse and showing the increase of velocity of transmission of the wave. This was done with one cannula inserted through the carotid into the aortic arch and a long cannula inserted at the femoral and run up the descending aorta to various measured distances from the top of the arch. The tracings suggest Frank's concept of the peripheral pulse as a recording of the central pulse by a poor manometer.

Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. LESTER R. DRAGSTEDT, JOHN VAN PROHASKA (by invitation) and HERMAN P.

HARMS (by invitation). Department of Surgery of The University of Chicago.

An extract was prepared from fresh beef pancreas and found to be effective in curing and in preventing the development of fatty infiltration and degeneration of the liver of depancreatized dogs, adequately treated with insulin. This extract which is fat free and soluble in 5 per cent NaCl solution was prepared in the following manner. Fresh beef pancreas was finely ground and extracted thoroughly three times with two volumes of 95 per cent alcohol. The alcohol filtrates were evaporated at room temperature and the dried residue extracted three times with several volumes of ether. The remaining fat-free residue was found to be effective when administered orally in amounts corresponding to 100 grams of fresh pancreas. The dried ether extract was not effective in a comparable dose.

Observations on the emetic, respiratory and certain other actions of strophanthidin in the cat. M. DRESBACH. Department of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

Our previous work (J. Pharmacol. Exper. Therap. **34:** 43, 1928) on the emetic action of strophanthidin showed that the heart cannot be the sole seat of this effect. Hanzlik and Wood (Ibid. **37:** 67, 1929), using pigeons, emphasized the liver as the locus of the emetic impulses aroused by digitalis glucosides. Our present studies indicate that in the cat the liver is not the only abdominal organ in which strophanthidin may possibly act to produce vomiting because practically complete denervation of it does not prevent the emesis. Experiments on hepatectomized animals have not yet been done in sufficient number to be decisive but work along that and other lines is being pursued.

It is noteworthy that doses of 0.20 to 0.35 mgm. per kgm. cause vomiting, usually within two to thirty minutes, when given by a saphenous vein, mesenteric vein, by direct injection into a lobe of the liver, and intraperitoneally, intramuscularly or subcutaneously. Profuse salivation, micturition, defecation and relaxation of the nictitating membrane may also result. Another striking effect often seen is an increase in respiratory rate from normal to 200 or 300 per minute, the tachypnea lasting in greater or lesser degree as long as an hour in some cats. Vomiting may be the only action noted or it may be accompanied by one or more, or all, of the other phenomena mentioned, when strophanthidin is given by any of the routes above named.

In our opinion, these facts, taken collectively, point to a central (medullary) action of strophanthidin rather than a peripheral one.

The emetic and respiratory actions of strophanthidin in the cat. M. DRESBACH. Department of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. (Motion picture demonstration.)

The picture will show that emesis is produced when strophanthidin is injected by any one of several methods, and that denervation of the liver does not prevent it. It will also show that an extraordinary tachypnea may also be produced by this substance when injected by various routes.

Results obtained in recent quantitative studies on lymph production. CECIL K. DRINKER and MADELEINE E. FIELD. The Department of Physiology, Harvard School of Public Health, Boston, Mass.

Making use of the technique described in the preceding paper it is possible to demonstrate the effects upon lymph flow and composition of the following manoeuvres: 1, increased and decreased tissue pressure; 2, increased venous pressure; 3, injections of horse serum.

Objective measures of hearing. H. DUBNER (by invitation), R. W. GERARD and H. KOBRAK (by invitation). Departments of Physiology and Otolaryngology, University of Chicago.

Evidence has been presented (Kobrak et al.) that cochlear potentials can persist after cochlear injuries sufficient to abolish middle ear reflexes. If impulses in the auditory nerve or paths were likewise eliminated it would indicate that cochlear receptor elements are not excited via the local potentials.

Apical lesions of one cochlea were produced in rabbits during an experiment or, aseptically, several days preceding; the other cochlea was fully destroyed. Cochlear potentials and middle ear reflexes were present before the lesion. A concentric electrode was placed in an auditory path (probably auditory thalamus) through a drill hole in the calvarium. When good responses of the brain to voice, tuning forks and metronome clicks were indicated by speaker and oscillograph the acute lesion was produced. Finally cochlear potentials and reflexes were again tested. In chronic experiments, auditory tracts were located by auditory stimulation with the crossed cochlea intact, and it was then destroyed.

In most experiments, acute or chronic, brain potentials were absent (chronic cases) or disappeared within fifteen minutes of producing the lesion, when reflexes, and usually cochlear potentials as well, were gone. (Loss of cochlear potentials, even in chronic cases, is anomalous and possibly related to loss of C. S. F. on entering the skull.) In at least two cases, however, auditory tract and cochlear potentials were clearly present two hours after producing a cochlear lesion that abolished the reflexes. Microphonic pickup and cochlear spread were excluded.

It follows that the middle ear reflex may be absent although impulses still ascend the auditory nerve. Since the reflex is normally elicited only by stimuli 20 to 40 decibels above threshold, this is not surprising. Perhaps after cochlear injury fewer impulses converge upon the motor cells, giving insufficient summation to evoke the reflex response, although some still enter the brain stem. Perforating lesions of the cochlea, in any event, abolish in order: middle ear reflexes, auditory path impulses, and cochlear potentials. With which, if any, conscious audition is to be equated is for further quantitative study to decide.

Studies on the energy metabolism of the hen. H. H. DUKES. Veterinary Research Institute, Iowa State College, Ames, Iowa, and Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y.

The Haldane gravimetric method of measuring the respiratory exchange was used. Determinations of the nitrogen metabolism were not made. Activity records were made in nearly all experiments. The general practice was to feed the hens 40 grams of corn at the beginning of the fast.

The effect of fasting on the R. Q. and the metabolic rate was studied in 50 experiments on 8 young hens (Plymouth Rocks) with the following average results:

HOURS AFTER FOOD	R.Q.	CAL./KG./HR.	CAL./SQ.M./HR. ($S = 0.10 \times W^{2/3}$)
1–5	0.96	3.18	41.99
22–29	0.74	2.80	36.51
44–53	0.70	2.58	33.60
72–78	0.71	2.36	29.94
88–101	0.71	2.30	30.74

In a larger group of older hens (32 Plymouth Rocks), 56 metabolism tests run 23 to 32 hours after food gave the following average results: R. Q., .73; Calories per kilogram per hour, 2.31; Calories per square meter per hour, 31.11. The lower metabolic rate in this group is probably an age effect.

Numerous observations in the interval between 24 and 29 hours after food show that during this time it is possible to repeat metabolic rate determinations on the same hen with accuracy.

In 37 determinations of the fasting metabolic rate at environmental temperatures ranging from 21° to 28°C. (average, approximately 24°C.), the heat loss due to vaporization of water varied from 12.5 to 22.8 per cent of the total heat loss. The average was 17 per cent. This is much lower than the average result obtained by Benedict, Landauer and Fox.

In the determinations just referred to, a positive correlation was found between insensible loss and metabolic rate. Although the correlation was not high, it was found to be statistically significant.

The effect of egg production on the fasting metabolic rate appears to be nil.

Intestinal motility in the horse: a motion picture. H. H. DUKES and J. SAMPSON (by invitation). Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y. (Demonstration.)

Several condemned army horses were used in this study. Although the animals were aged, their general health was good. The horses were anesthetized with chloroform; or were subjected to spinal transection and brain destruction under chloroform anesthesia, following which artificial respiration was used. The abdomen was then opened and intestinal motility studied. The exposed intestine was frequently bathed with warm physiological salt solution. At times the more pendulous portions of the intestine were allowed to float in warm saline in a large tub. Motion picture records reveal the following types of motor activity.

Small intestine. Pendular movements are common. Their frequency appears to be less than in the rabbit. At times what appear to be rhythmic segmentations accompany the pendular movements. Rhythmic segmentations not accompanying pendular movements are clearly present in the small intestine. No peristaltic rushes were observed.

Large intestine. Near the base of the cecum is seen a wave running over the saculations. Haustral contractions in the great colon, the small colon, and the cecum are evident. What appears to be a propulsive movement is also seen in the great colon.

Simultaneous facilitation and extinction of motor response to stimulation of a single cortical focus. J. G. DUSSER DE BARENNE. Laboratory of Neurophysiology, Yale University School of Medicine.

Repetition of faradic stimulation of a focus of the motor cortex with an interval of a few seconds results in augmentation of response, facilitation. In 1934 the present author and W. S. McCulloch reported that electrical stimulation of a single motor focus results in its temporary inactivation, as shown by the reduction or absence of response to an appropriately timed second period of stimulation. The interval at which this extinction appears depends upon the experimental conditions; in the anesthetized animal it is usually found to be from 13 to 20 seconds.

As facilitation and extinction are obviously antagonistic factors in central neural activity, it became of interest to study facilitation during extinction. This was done in the following way. Three electrical stimulations of equal strength and duration were applied to a motor focus, the first as the extinguishing stimulation, the second after an interval of 13 to 20 seconds, the third 2 to 3 seconds after the second stimulation. The response to the third stimulation was larger than that to the second, often even larger than that to the first stimulation This shows that the third response, following the second stimulation after an interval of 2 or 3 seconds, is facilitated by that stimulation. If only two stimulations, 2 of 3 seconds apart, were given, the facilitation to the second response was much greater. That the facilitation in the first experiment, with three stimulations, was less must be due to the fact that the motor focus was then still under the extinguishing influence of the first stimulation.

Thus it can be shown: 1. that facilitation and extinction can exist simultaneously in the central nervous system upon cortical stimulation, and 2. that, when present simultaneously, they are acting antagonistically in determining the size of motor response to repeated electrical stimulation of a single motor focus. (See also the abstract by W. S. McCulloch and J. G. Dusser de Barenne on p. 99 of these Proceedings.) (Lantern slides.)

Conditioning neurosis treated by sedatives. S. Dworkin, W. Bourne (by invitation) and B. Raginsky (by invitation). McGill University, Montreal, Canada.

Two dogs in the course of conditioning experiments developed a permanent neurosis of the inhibitory type. The animals thus affected failed to respond to any of the previously positive and negative signals, they refused to take food in the conditioning room, and evinced various signs of fear. On these particular animals we studied the effects of five drugs (alcohol, nembutal, avertin, sodium amytal, sodium bromide) which we had previously observed to exert on normal animals a certain degree of disinhibiting action. The first four all produced a distinct disinhibition, in which, however, differentiation seldom reappeared. The effect, too, was evanescent, for within twenty-four hours the animals had resumed the inhibited state. The effect of sodium bromide, administered daily, was quite different. Within ten days the neurosis subsided and differentiation was re-established. Administration now ceased. At the end of three months the animals were still as good as normal. We have therefore confirmed Pavlov in showing that sodium bromide has an action on the higher centers that is much more permanent than any of the other drugs.

The specific dynamic action of glycine intravenously administered to nephrectomized dogs. A. G. Eaton, Shirley C. Cordill (by invitation) and

J. L. Gouaux (by invitation). Department of Physiology, Louisiana State University Medical Center, New Orleans, Louisiana.

Six dogs anesthetized with sodium amytal were given 10 grams of glycine, dissolved in 115 cc. 0.9 per cent sodium chloride, intravenously after three determinations of the basal metabolism. Although these animals deaminized somewhat less glycine than trained unanesthetized dogs, the average increase was 12.75 calories or 0.22 calorie per millimol of glycine deaminized, which results are in agreement with the experiments of Wilhelmj, Bollman and Mann. The R. Q. rose from 0.782 to 0.813. Thus it is practicable to study the specific dynamic action of glycine in dogs anesthetized with sodium amytal.

Three determinations of basal metabolism were made on fourteen other dogs, a bilateral nephrectomy was done, three further basal metabolic determinations were made, glycine injected intravenously and the metabolism followed for five hours as in the control series described above. In these animals 7.88 grams of glycine were given because the average excretion of glycine in the urine of the control series was 2.12 grams. The average specific dynamic action of the glycine was 14.74 calories. The R. Q. rose from 0.770 to 0.818; blood urea nitrogen from 12.5 mgm. per cent before injection of glycine to 40.1 mgm. per cent at the end of the five hour period. As a further control three nephrectomized dogs were studied following the injection of 115 cc. of 0.9 per cent sodium chloride. The average increase in heat production was 1.30 calories.

So far as we are able to determine, the kidneys play no part in the specific dynamic action of glycine.

Sounds due to skeletal muscle contraction. R. W. Eckstein (introduced by C. J. Wiggers). Western Reserve University, School of Medicine.

Participation of muscle contraction in production of the first heart sound is generally admitted but occasionally questioned (cf. Dock: Arch. Int. Med. **51:** 737, 1933). A first approach to the question was made by registering sounds from a frog's gastrocnemius muscle contracting under various degrees of isometricity up to complete abolition of shortening.

A segment capsule 2 cm. in diameter covered with a tightly stretched membrane and supporting a short bamboo lever stitched to the muscle was used for sound pickup. It was connected by tubing to a modified Wiggers-Dean capsule.

These modifications which reduce its shortcomings were: 1. The rubber cement membrane was vulcanized thereby increasing its permanence and stretch. 2. The sensitivity was increased by using a convex mirror which projected light 2 to 3 meters thus allowing the side tube to be left open with complete elimination of impacts. 3. The periodicity was reduced by placing damping plates both in front and behind the membrane. The frequency of the entire system (receiver, tubing, and recorder) was 290 to 350/sec.

Results show that an absolutely isometric contraction causes no sounds but when shortening of 166 micra is permitted small vibrations occur. At shortening commonly considered isometric, vibrations were found from the moment of contraction to its maximum. Under conditions still isometric (shortening of 416 micra) small vibrations even occur during relaxation. The numbers and intensity of the vibrations depend upon the vigor of contraction as tested on muscles in various stages of fatigue.

Since the periods of the vibrations ranged from 0.0124 to 0.008 while that of the system was 0.0029, the vibrations must originate in the muscle and not in the system.

The conclusion is reached that muscle contracting under so-called isometric conditions gives rise to vibrations having frequencies of wide range and resembling those of the first heart sound.

A hemidecerebrated cat preparation, with observations. LEON ECTORS[1] and NORRIS L. BROOKENS (introduced by R. W. Gerard). Department of Physiology, University of Chicago.

It proved rather simple (little hemorrhage or fall of temperature or blood pressure in several hours) to remove one lateral half of the cat's encephalon rostral to the midbrain. The structures of the median plane and about the third ventricle are clearly exposed and available for local stimulation, drug application, etc.

At rest, such an animal showed regular and strong heart beat and respiration with occasional panting. Leg muscle tone and patellar, achilles and corneal reflexes were symmetrical and normal. Subcutaneous temperature was 36° in both uncovered hind legs. On the operated side (left) the pupil was widely dilated, the other semislit, and usually neither responded to light. (Both optic nerves were intact.) The right nictitating membrane was more retracted than the left.

Pinching any leg gave specific contraction of that limb and a non-specific anger reaction: bared claws and teeth, whisker retraction (possibly more on the left) and widening of the palpebral fissure, retraction of the nictitating membrane and pupil dilation on the right.

Direct stimulation (induction shocks) of the median structures yielded typical crossed motor effects from the frontal cortex, nothing striking from the thalamus or callosum. Extensive and specific responses were obtained from various hypothalamic regions. The supramammillary region evoked running movements and characteristic rage; with eye and facial responses, increased blood pressure and respiration, and bilateral erection of the back and tail hairs. The supratuberal region gave the rage responses without running but, in the anterior portion, with snarling. Hissing, licking tongue movements, salivation, micturition and tail lashing were also obtained in basal regions. The supraoptic region evoked no rage but rather caused relaxation. The paraventricular gray gave only pupil dilatation, more caudal regions (highly localized) gave either constriction or dilatation and a characteristic scratching by the ipsilateral legs and sudden maintained dorsolateral flexion of the neck, likewise to the ipsilateral side. Further studies are in progress.

Further observations on cortico-adrenal extracts.[2] M. EHRENSTEIN (by invitation) and S. W. BRITTON. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Normal estrous cycles are suppressed in adrenalectomized rats usually within twenty-four hours and at latest three days after operation. Life-maintaining extracts obtained from cattle, cow or bull adrenal glands readily restored estrous activities to normal. Estrogenic substances such

[1] Belgian Fellow of the C. R. B. Educ. Found.

[2] Aided by a grant from the Rockefeller Foundation.

as the benzoic acid ester of the dihydro-follicular hormone ("Progynon-B," Schering Corp.) were able to keep adrenalectomized rats in normal estrus only when given in extremely large doses; usually 100 rat units daily were necessary. Furthermore it appeared that cow as well as bull adrenal glands do not contain any of the specific sex hormones, i.e., estrogenic substances (effective on oöphorectomized animals) or male sex hormones (capon comb-growth influencing).

By a procedure similar to the isolation methods for gonadotropic substances, there was obtained from mixed cattle as well as separate cow and bull adrenal glands an extract which produced a marked effect on the growth of ovaries of immature rats. The average weight of ovaries in the experimental animals was 36 per cent higher than that of the controls, and the average percentage ovary-weight/body-weight was 50 per cent higher. Preliminary histological examination yielded evidence of the presence of mature follicles in the experimental animals.

Some effects of replacement of ions in a diet poor in inorganic salts. ERCEL S. EPPRIGHT (by invitation) and ARTHUR H. SMITH. Department of Physiological Chemistry, Yale University. (Read by title.)

Albino rats were given a low-salt diet plus various inorganic supplements, most of which represented portions of the Osborne and Mendel salt mixture in quantities normally consumed by rats. Of all supplements given, calcium and phosphorus proved most efficacious in maintaining satisfactory nutritional state, growth, and intestinal flora characteristic of normal rats.

Addition of calcium, calcium and phosphorus, sodium chloride or sodium chloride plus potassium, ferric chloride, ferric citrate, or hydrochloric acid not only failed to prevent but in many cases augmented the hypertrophy of adrenal glands observed on the low-salt diet. Calcium, phosphorus, and magnesium together, or calcium alone in restricted amounts, greatly reduced the growth of the glands. Sodium chloride or sodium chloride plus potassium stimulated the growth of hepatic and cardiac tissues.

Modifications of dietary salts were reflected in the composition of tissues. Excepting kidneys and adrenal glands, a preponderance of calcium usually promoted dehydration; a preponderance of sodium, excessive hydration. Directly, or indirectly, limitation of ions to sodium and potassium, in contrast to calcium and phosphorus, reduced the tissue lipids, particularly in skin. Calcium, possibly aided by related elements, influenced more profoundly the potassium and sodium contents of muscle than did these elements themselves. The ratio of sodium to potassium in muscle tissue, much distorted on the sodium or potassium regimes, was exactly normal when calcium was abundant, even though sodium and potassium were withheld. Potassium and sodium were mutually antagonistic toward their retention in muscle. Diminished potassium and augmented sodium generally accompanied poor nutritional states. Chloride content did not follow shifts in base but varied directly with the dietary level.

Studies on the coronary blood flow. H. E. ESSEX, J. F. HERRICK, E. J. BALDES and F. C. MANN. Divisions of Experimental Medicine and Physics and Biophysics, Mayo Clinic, Rochester, Minnesota.

Further studies have been made on the blood flow in the circumflex

branch of the left coronary artery of the intact dog by use of the thermo-Stromuhr method of Rein. As in previously reported experiments under the influence of exercise on a treadmill the flow is markedly increased. The inhalation of amyl nitrite following cocainization of the nasal passages results in a marked increase in coronary flow. Thyroxine augments the coronary blood flow as much as 300 to 400 per cent over the control values.

Stimulation centrally of the sectioned vagus nerve in experiments on the dog under sodium amytal anesthesia results in a marked increase in coronary blood flow which is accompanied by the usual rise in blood pressure, the pulse rate remaining constant. The effect of stimulation of other vasomotor nerves on the coronary blood flow is being investigated in the anesthetized dog.

Effects of repeated and prolonged stimulation of the vagus nerve in the dog. G. H. ETTINGER, G. E. HALL and F. G. BANTING (introduced by V. E. Henderson). Department of Medical Research, University of Toronto, Toronto, Canada.

The vagus nerve was stimulated continuously in fifteen dogs, for periods of 2 to 7 hours, under dial or nembutal. This process was repeated daily or at intervals of 2 to 7 days, as long as the animals survived.

Two methods of stimulation were used: a, the method of Chaffee and Light, in which currents were induced in a small coil imbedded in the neck, the terminals being wrapped around the vagus nerve; b, the method of Cannon, in which insulated gold-platinum wires wrapped around the nerve were allowed to protrude through the neck where they were attached to the secondary of an induction coil.

In experiments lasting up to periods of 200 days with a total vagus stimulation of as long as 332 hours, we failed to produce any degenerative lesions of the myocardium or coronary arteries such as were described by Hall, Ettinger and Banting after repeated and prolonged administration of acetylcholine.

Capillary bleeding from the stomach and small bowel was common, during and after stimulation, but no acute or chronic ulcers were produced. Most of the animals died of pneumonia.

Uterine motility in the cow. EVERETT I. EVANS and FRED W. MILLER (by invitation). U. S. Dept. of Agriculture.

The motility of the uterus of the cow has been followed by the balloon-manometer method, throughout the oestrous cycle. On the day of oestrus, the activity is very marked and the injection of pituitrin causes marked spasm of the uterus. About 30 to 36 hours after the onset of oestrus, ovulation occurs, after which the uterus is relatively refractive to pituitrin. However, the uterus of the cow, unlike that of the rabbit, shows considerable "normal" activity for some 2 to 3 days after ovulation, although refractive to pituitrin.

Spontaneous activity decreases until the 11 to 16th day after ovulation, during which time the uterus is relatively refractive to pituitrin. From the 16th to 21st day, the uterus becomes more active spontaneously and reacts more strongly to pituitrin. On the 21st day (when oestrus usually appears again) uterine motility is very marked, as is the uterine response to pituitrin. The cycle of events, as stated above, is then repeated.

Ovariectomy abolishes any motility of the uterus in the cow. The spontaneous motility of the cow's uterus, in relation to ovarian changes

during the oestrous cycle, resembles that described by Reynolds for the rabbit, except for the motility of the uterus immediately before and for 2 to 3 days after ovulation.

The effect of prolactin upon the milk yield of lactating cows and goats. EVERETT I. EVANS. U. S. Dept. of Agriculture. (Read by title.)

Administration of an alkaline extract of beef anterior pituitary in amounts assaying to total daily dose of 450 pigeon units of prolactin, increased greatly the milk yield of lactating cows and goats. This increase in some cases amounted to 25 to 50 per cent of the level immediately preceding the injection period. Following the cessation of injections, the milk yield immediately returned to the levels obtaining before the injections.

No success was experienced in bringing back into lactation 2 cows that had been allowed to "go dry" for this purpose. Ovariectomy does not alter the response of the lactating cow to injected prolactin.

The cost of the injected material precludes its application to the problem of increasing the milk yield of dairy animals. Further, the fact that the increased yield did not carry over after the injections had been stopped would make it appear that increase in milk yield could better be obtained by application of sound genetic principles in breeding rather than hormone therapy.

Ovulation, fertilization and early development of the mammalian egg. EVERETT I. EVANS. U. S. Department of Agriculture. (Cinemicrographic demonstration.)

A two-reel motion picture has been prepared which shows by normal speed and time-lapse cinemicrography the bursting of the follicle, the attack of the ovum by spermatozoa, and the early development (in tissue culture) of the rabbit ovum through to the blastocyst stage. It is intended that this film be used for teaching some of the fundamentals of the physiology of reproduction. Rental or purchase of copies of this film for use by teachers of the biological sciences may be made by application to the Division of Motion Pictures, U. S. Dept. of Agriculture, Washington, D. C.

A simplified photoelectric colorimeter using light filters. KENNETH A. EVELYN (introduced by J. B. Collip). University Clinic, Royal Victoria Hospital, Montreal, Canada. (Demonstration.)

The instrument is a photoelectric colorimeter of the single photocell and galvanometer type, which has been designed not only for routine biochemical colorimetry, but more particularly for the colorimetric analysis of solutions containing more than one colored component.

Two models, a portable and a high sensitivity, will be demonstrated. The internal design and construction will be illustrated by *1*. A partially assembled instrument and *2*. A median vertical section cut through the body of the colorimeter. The operation of the apparatus and its application to colorimetric determinations in the presence of interfering colors and precipitates will be illustrated by demonstrations of the following procedures:—

1. The analysis of solutions containing oxyhemoglobin together with its derivatives such as reduced hemoglobin, carbon monoxide hemoglobin and methemoglobin.

2. The determination of the pH of colored solutions, including human serum.

3. The determination of bilirubin in serum and stool.

Nature of R and T potentials in the mammalian heart. J. A. E. EYSTER, M. R. KRASNO (by invitation), C. A. MAASKE (by invitation) and M. J. ULEVICH (by invitation). University of Wisconsin.

The methods employed in these experiments were similar to those previously reported in the work on tortoise and frog hearts. The isolated heart of rabbits and cats under artificial perfusion were placed at the center of a circular disc conducting field and potential-time curves were recorded from the field at a constant distance along various axes around the heart. From the potential distribution, the nature and distribution of the electrical charges in the heart responsible for the R and T potentials are determined. As in the case of the tortoise and frog, the electrical charges are arranged as dipoles and the R and T potentials can be explained to a high degree of approximation by the assumption of two asynchronous dipoles for each set of potentials.

Plasma colloid osmotic pressure as a factor in edema formation and edema absorption. GEORGE FAHR and ARTHUR KERKHOF (by invitation). Department of Medicine, University of Minnesota.

Edema appears when the colloid osmotic pressure of the blood plasma is reduced in nephritis through loss of the plasma proteins and in normal dogs when the colloid osmotic pressure is reduced by removal of a large proportion of the plasma proteins by plasmaphoresis. In experimental animals a high grade anasarca and massive ascites with a retention of as much as 3800 cc. of water can be produced in 24 hours after the beginning of the experiment If gum acacia in sufficient amount is injected into patients with lowered colloid osmotic pressure to bring the colloid osmotic pressure up to about 15 or 16 mm. of mercury, the edema disappears concomitant with a greatly increased urinary output. No edema will be produced in plasmaphoresis on dogs if gum acacia is substituted for the proteins removed from the plasma and the colloid osmotic pressure of the plasma thereby brought up to approximately 15 mm. of mercury. The colloid osmotic pressure of the plasma is therefore an important factor in edema formation and edema absorption in those conditions wherein plasma proteins are reduced to about half the normal or lower, much as Starling predicted in 1899.

Effect of nicotine on the oxidations of the diabetic brain. J. F. FAZEKAS (by invitation) and H. E. HIMWICH. Department of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

We have previously demonstrated that nicotine inhibits the oxidation of lactic acid but not of glucose in the brain of normal rats, cats and dogs. This work was continued on diabetic brains in an effort to elucidate the oxidative mechanisms of that organ in diabetes. It is well known that the normal brain has an R. Q. of unity, which indicates the oxidation of glucose or lactic acid. Since the diabetic brain also has an R. Q. of 1.00, it became of interest to determine whether this organ could oxidize glucose in diabetes.

The O_2 consumption of brain tissue excised from diabetic dogs and cats

was determined in the Warburg respirometer. The concentration of nicotine was 0.014 M.

The data reveal that in the diabetic as well as the normal brain, nicotine inhibits the oxidation of lactic acid but not of glucose. We therefore conclude that the diabetic brain may oxidize glucose.

A method for prolonged stimulation of the nervous system.[1] FREDERICK A. FENDER[2] (introduced by J. C. Hinsey). Department of Surgery, Stanford University School of Medicine. (Demonstration.)

The publication of a preliminary note upon the "remote control" principle by Light and Chaffee found Dr. W. J. Merle Scott of Rochester University School of Medicine and the author with a device of similar type already in an operating stage. The last two investigators must, we feel, be given complete credit for originating the method. However, we felt that the apparatus as developed at Stanford should be demonstrated because its relative simplicity may lead to wider use in other laboratories than that accorded the relatively complex and expensive apparatus of Light and Chaffee.

The principle is that of the induction coil. Six primary coils are arranged on the faces and at the ends of a shell, which is incorporated into a cage. These set up electromagnetic fields in the three primary planes when energized, in pairs, successively, by alternating current. Distribution of the current is carried out by a motor-driven switch. The ordinary 60 cycle, 110 or 220 volt power source is used.

The secondary units, in which the stimulating electromotive force is induced, are small coils of 6000 turns, coated with moulded rubber, fitted with solid or braided silver leads (depending upon the motion to be encountered). They are inserted surgically into the body of the subject, with leads in contact with the nervous tissue to be stimulated. After recovery, repeated observations on the effect of stimulation may be made, or the subject may be submitted to prolonged stimulation.

Working models of the apparatus were demonstrated. Photographs of the device as used in our laboratory were exhibited, giving the details of construction for those who may care to duplicate the set-up.

Experimental results obtained, through the use of the method, in stimulation of the lumbar sympathetic trunk, the thoracic sympathetic trunk, and of some portions of the cerebral cortex in the dog, were shown graphically.

Electrolyte changes in rat muscle during stimulation. W. O. FENN. Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

One leg of an urethanized rat was stimulated through the sciatic nerve, usually for half an hour, at a frequency of 400 condenser charges per minute. Immediately after the close of stimulation, corresponding muscles of the hind legs were sampled and subsequently analyzed for potassium, sodium, chloride, phosphorus, calcium, magnesium, and water. Changes in calcium, magnesium and usually phosphorus between the stimulated and resting muscles were negligible. Stimulated muscles showed a loss of potassium averaging 6.1 m.-eq. per 100 grams dry weight,

[1] Entirely financed by grants from The Committee on Scientific Research of The American Medical Association.

[2] Fellow of the Rockefeller Foundation.

which was balanced by a gain in sodium of 8.3. The sodium gain in excess of the potassium loss was approximately equal to the average gain in chloride, amounting to 2.8 m.-eq. The stimulated muscles also gained water, half the water being accounted for by the chloride in the tissue spaces, the other half being inside the cells. During a 2-hour recovery period the muscles regained most of the potassium lost and lost most of the sodium, chloride and water which they had gained. The essential change is therefore an exchange of sodium for potassium during stimulation which is reversed in recovery.

Cephalin and corneal extracts in blood coagulation. JOHN H. FERGUSON. Department of Physiology and Pharmacology, University of Alabama.

Controlled experiments on the clot facilitating powers of cephalin on a, benzene-extracted (dog) plasmas; b, Berkefeld-filtered plasmas, and c, purified fibrinogen and prothrombin mixtures (devoid of anti-thrombic factors) lend support to the hypothesis (Bordet: Mills) that cephalin as well as calcium is an essential factor in the *direct* activation of the coagulant. Cold benzene extraction readily renders the above systems incoagulable on simple recalcification. Boiled saline suspensions of the evaporated benzene extracts clot the recalcified reagents. Purified brain cephalin in high dilution acts similarly, and evinces a remarkable capacity to restore a high grade of thrombic activity to weak Ca-prothrombin prepared from platelet-free plasmas. Washed platelet suspension acts a, as thrombin-activator, and b, directly upon recalcified, prothrombin-free fibrinogen.

Dogs' corneae (chosen because of their avascularity) can be dried in an air current, stored in a dry container, and prepared for use as a saline extract. Such extracts clot recalcified fibrinogen (prothrombin-free) and hasten the coagulation of fibrinogen plus calcium plus prothrombin mixtures. Without raising the academic question of the identity of blood- and tissue-"thrombins" (A. Fischer), it may be asserted on the basis of these experiments that the coagulant activity of corneal extracts exemplifies the possibility of preparing a coagulant factor from blood-free tissues.

Carbamino compounds of CO_2 with human hemoglobin. J. K. W. FERGUSON. Department of Physiology, University of Western Ontario, London, Canada.

The chemical method of Ferguson and Roughton (1934, J. Physiol. **83**) has been applied, with slight modifications, to solutions of human hemoglobin. Combined CO_2 other than carbamino CO_2 are precipitated with alkaline $BaCl_2$. The $BaCO_3$ thus formed is removed by centrifuging, and the carbamino CO_2 ($HbCO_2$) remaining in the supernatant is measured by the Van Slyke technique. When using human hemoglobin it was found necessary to add with the alkaline $BaCl_2$, a volume of 0.4M Na_2CO_3 equal to that of the hemoglobin solution, to increase the bulk of the precipitate and thus to secure satisfactory removal of the $BaCO_3$ on centrifuging.

The rates of dissociation of $HbCO_2$ in solutions of human and ox hemoglobin have been investigated at temperatures of 37°C., 18°C., and 1°C. From these rates, the approximate loss of $HbCO_2$ which occurs during an ordinary determination can be calculated. It appears that about 33 per cent of the $HbCO_2$ is lost. Accordingly, observed figures must be

multiplied by a correction factor of 1.5. A correction of about 1.2 in magnitude should be applied to the published results of Ferguson and Roughton, and to their estimate of the importance of $HbCO_2$ in the transport of CO_2.

The capacity of human hemoglobin to form carbamino compounds has been found to be about 20 per cent greater than that of ox hemoglobin under comparable conditions.

At 37°C. and physiological pH and pressures of CO_2, $[HbCO_2]$ in solutions of human hemoglobin (as in ox hemoglobin), varies only slightly when the pH is varied by changing only the pressure of CO_2 (from 5 mm. Hg to 85 mm. Hg). Thus the dissociation curve of $HbCO_2$ is practically flat. On the other hand, $[HbCO_2]$ varies inversely as the degree of oxygenation of the hemoglobin;

$$\frac{\Delta [HbCO_2]}{\Delta [O_2]} = -0.23 \pm 0.05$$

Hence, the significance of $HbCO_2$ in the transport of CO_2 can be calculated from the R. Q. at any moment. For example if the R. Q. were 0.8, the per cent of the expired CO_2 which had been carried as $HbCO_2$ would be $\frac{0.23}{0.8} \times 100 = 29$ per cent.

A quantitative method for the collection of lymph. Madeleine E. Field and Cecil K. Drinker. Department of Physiology, Harvard School of Public Health, Boston, Mass.

Apparatus will be described and illustrated whereby lymph may be collected from popliteal lymphatics in the dog under constant conditions of mechanical massage. The arrangement of the experiment makes it possible to make the following measurements and alterations in experimental conditions: 1, flow and composition of lymph; 2, venous pressure in the part producing lymph; 3, increased and decreased tissue pressure; 4, increased venous pressure; 5, altered arterial pressure; 6, anoxemia and other chemical changes in the blood.

"Hunger" as a factor determining the magnitude of conditioned and unconditioned salivary responses. G. Finch (introduced by W. Horsley Gantt). National Research Fellow in the Conditional Reflex Laboratory, Phipps Psychiatric Institute, Johns Hopkins University.

Dogs in which conditioned salivary responses had been thoroughly established were tested after food-deprivation periods ranging from 0 to 96 hours. The data show that the magnitudes of both the unconditioned and conditioned responses vary with food-deprivation time.

This result is consistent with the findings from studies of other learning phenomena—despite the fact that the criteria used in measuring the strength of conditioned reflexes are not obviously analogous to the criteria used in measuring other learning.

The hypophysis and menstruation. W. M. Firor and C. G. Hartman. Johns Hopkins Medical School.

An effort was made in 10 Macacus rhesus monkeys, first, to remove all of the hypophysis; secondly, to remove all except the posterior lobe; and

thirdly, to remove all except the pars intermedia and the posterior lobe. The animals were then injected with Amniotin of known potency, and the presence or absence of bleeding was determined by daily vaginal washings. The results seem to show that the posterior lobe is necessary for bleeding to occur following Amniotin injections. The actual contents of the sellae were ascertained by serial sections.

The action of a single vagal volley and the dependence of its chronotropic effect on the rhythmic mechanism of the pacemaker. ERNST FISCHER (introduced by W. O. Fenn). Medical College of Virginia, Richmond, Va.

The different chronotropic effects of single vagal volleys reaching the heart at various times of the cardiac cycle cannot be explained by the simple assumption of the formation of a definite amount of A. C. substance at a fixed rate and by its disappearance in consequence of diffusion and of destruction by esterase. Particularly in experiments on the eel heart it becomes evident: a, that a vagal impulse, reaching the pacemaker after one third of the time needed for a normal cycle has elapsed since the last beat, causes a large inhibiting effect on the present cycle and steadily declining effects on the two to four subsequent cycles; b, that a vagal impulse, reaching the pacemaker about the time of a normal beat, has no influence or only little on the next cycle, but may inhibit the later cycles more or less markedly. An analysis of these experiments suggests the assumption that the state of rhythmic activity of the pacemaker at the moment of the arrival of the vagal volley determines not only the sensitivity of the pacemaker against A. C. substance, but influences as well the rate of formation and to a less degree the amount of A. C. substance freed.

The dromotropic action seems to depend in a similar way on the heart cycle. But the inotropic and the bathmotropic action show no dependance on the cycle, and their respective curves of accumulation and disappearance of A. C. substance exhibit time characteristics quite distinct from those of the chronotropic action. The chronaxie for the chronotropic and the inotropic effect is about the same, but the time-intensity curves are distinctly different. All this evidence favors the assumption that at least the chronotropic and the inotropic action of the vagus are conveyed by different fibers.

The respiratory system which maintains the heart rate in embryonic fish. KENNETH C. FISHER (introduced by L. Irving). Departments of Biology and Physiology, University of Toronto.

The heart beat of embryonic salmon and trout is reversibly slowed by lack of oxygen and by the respiratory poisons cyanide and carbon monoxide. The retardation is not instantaneous and the delay is attributable to the utilization of energy reserves created by the unpoisoned oxidative system in the presence of oxygen. The time of retardation and recovery measures the quantity of reserve which is available when oxidation is restricted by treatment with cyanide or carbon monoxide.

The effects of carbon monoxide, cyanide and oxygen lack are not identical. Oxygen lack slows the heart gradually and steadily while carbon monoxide and cyanide slow the heart rapidly to about sixty per cent of its normal rate and this retarded rhythm may then remain nearly steady for

several hours. This steadily maintained rate is absent in the oxygen lack experiments and hence is associated with the presence of oxygen.

That portion of the rate which is sensitive to carbon monoxide and cyanide is affected within thirty minutes. The retardation by carbon monoxide is entirely removed by light in about seven minutes. Diffusion can be no part of this latter delay and it is attributable to the gradual restoration of a depleted reserve.

The results indicate the nature of the dependence of the heart rhythm upon oxidation. Energy derived from oxidation stands in close relation to the heart rhythm as is shown by the comparative speed of the light effect on the carbon monoxide poisoned preparation, but it is evident that the carbon monoxide poisoned reaction is distinct from the actual rhythm. The extent of the delay indicates how close this chemical reaction lies to the final physiological process.

When oxidations are impaired by the presence of cyanide or carbon monoxide, energy for the partial maintenance of the normal rhythm is supplied from a system utilizing oxygen (but insensitive to carbon monoxide and cyanide) and probably also from an anaerobic mechanism. During oxygen lack an energy store is available which is built up in the presence of oxygen. This store in anaerobiosis passes through the system which can be inactivated by the use of cyanide or carbon monoxide.

Depression of the cochlear response in order of frequency.[1] EDMUND P. FOWLER, JR. and T. W. FORBES (introduced by H. B. Williams). Department of Pathology and Otolaryngology, College of Physicians and Surgeons, Columbia University and N. Y. State Psychiatric Institute.

Action currents were recorded in cats under barbiturate anesthesia from the round window using the degree of amplification necessary to bring the response to the observer's auditory threshold as a measure of intensity. The opposite ear was used as a control. Stimulus tones covered the range from approximately 256 to 10,000 cycles. Agents were applied to the round window membrane.

Sodium chloride and calcium chloride, quinine dihydrochloride powder and pure glycerine produced a loss which appeared first in the higher tones and progressed regularly to the lower tones. The rapidity and degree of loss were in general proportional to the three concentrations used (crystals, 25 per cent and 10 per cent). Loss as great as 40 to 70 decibels was obtainable and no recovery has been noted to date with salt solutions. Dextrose crystals, 1 per cent sodium chloride and distilled water produced little or no loss.

A microscopic examination of sections showed a progressive degeneration of hair cells which was most marked in one animal which survived for the longest period (3 weeks). The histological picture is identical with that in the deafness of old age, drug poisoning and stimulation deafness.

Our results are interpreted as due to the effect on end organs of an increased concentration of sodium or calcium which travels toward the apex of the cochlear spiral and they are therefore in line with a "place" theory, as are reports by Davis et al., and by Culler, with other techniques. It is suggested that our agents interfered with formation or liberation of a

[1] The work was aided by grants from the Research Council of the American Otological Society and the Hayden-Coakley Fund.

chemical mediator and its access to the nerve ending by the increase or reduction of membrane permeabilities. The variation of cell permeability with increased sodium and calcium demonstrated by Osterhout, et al.; evidence for a chemical mediator reported by Derbyshire and Davis; and our degeneration of hair cells after an interval, favor this view.

The fact that the first demonstrable degeneration occurs in the hair cells supports the contention of those who postulate that the cochlear effect originates in the hair cells.

The peripheral blood flow in surgical shock. NORMAN E. FREEMAN, JOHN L. SHAW and JOHN C. SNYDER (introduced by W. B. Cannon). Harvard Medical School.

The blood flow through the hand at controlled temperatures was determined in clinical cases of surgical shock. A reduction in circulation was found. The effect of certain stimuli on the circulation in the hand was then studied. Cold, pain, fear and asphyxia, recognized to be shock-producing stimuli, caused a decrease in the volume flow. Studies were also conducted during operations. Traumatic stimuli diminished the circulation in patients even under general anaesthesia.

The slow rate of flow in cases of shock was found to be associated with a low oxygen saturation of the venous blood. Where the condition of shock had persisted, the lowered carbon dioxide combining power of the blood attested to the acidosis from asphyxia of the tissues.

The reactive hyperemia after release of a tourniquet was found to be slight and of short duration. This abnormality indicated the inability of the tissues to obtain an adequate supply of blood.

Studies previously reported on the effect of temperature on the volume flow of blood in the hand indicated a dual control. Through the sympathetic vasomotor nerves, the circulation is modified in accordance with the needs of the body as a whole. After sympathectomy, the blood flow varies with the metabolic demands of the tissues.

The peripheral blood flow in cases of shock is diminished probably through vasomotor impulses which originate from various traumatic stimuli. The circulation may thereby be shunted to more essential organs, but at the same time the peripheral tissues become deprived of a supply of blood necessary for their metabolism. The venous anoxemia and acidosis indicate the severity of this deprivation.

Previous studies showed that the blood volume in experimental animals was reduced through prolonged hyperactivity of the sympathetic nervous system. Vasoconstriction with increased capillary permeability from tissue asphyxia was considered to be the dominant factor in this process. The present investigations on patients indicate in part the mechanism through which the peripheral tissues may become asphyxiated. The hypothesis suggested by Erlanger and his collaborators is supported—that surgical shock is the clinical manifestation of a diminished circulation.

The influence of irradiated ergosterol and parathyroid extract upon the rate of disappearance of intravenously injected calcium chloride. SMITH FREEMAN (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois. (Read by title.)

These experiments were intended to demonstrate whether or not parathyroid extract or irradiated ergosterol influenced the rate of removal of calcium from the blood stream. To accomplish this end it was also necessary to establish the uniformity of the rate of removal of injected calcium from the circulating blood of normal dogs.

Healthy adult dogs were used ranging in weight from 12 to 20 kgm. Their stock diet consisted of cooked ground meat, bone meal, and white bread. During the week of irradiated ergosterol administration the dogs received a diet of cooked corn-meal and white bread, a diet which has been shown to have a low calcium content. All injections were carried out 24 hours after the last feeding.

Six normal dogs were injected twice, the same dose (10 or 15 mgm./kgm.) being used for each injection; the second injection was given 1 week after the first. The results showed a practically constant rate of disappearance of injected calcium in any given animal.

The normal rate of calcium disappearance was determined in 8 more dogs and it was found that the serum calcium was practically normal 6 hours after injection of 10 mgm. of calcium per kgm. body weight. Seven of these dogs were given 12 units per kgm. of parathyroid extract subcutaneously and 18 hours later were injected with the same dose of calcium. The results show a definite prolongation of the serum calcium elevation in six of the seven dogs injected.

Six dogs were placed on a low mineral diet and were given 2 doses each of 5,000X Viosterol; the dosage was 0.10 cc. per kgm. body weight. Seven days later calcium injections were made. The results of this experiment show without exception a prolongation of the hypercalcemia as compared to the normal rate of calcium removal and demonstrate a similarity in the influence of parathyroid extract and irradiated ergosterol upon the rate of disappearance of calcium from the blood stream.

The influence of an inorganic salt mixture on the rate of tooth eruption in the thyroparathyroidectomized and unoperated albino rats. B. J. Frey (by invitation), Valeria Genitis (by invitation), Isaac Schour (by invitation) and R. D. Templeton. Department of Dental Histology, University of Illinois and the Department of Physiology, Loyola University School of Medicine.

The results here summarized were obtained on 53 albino rats taken from the Loyola colony. Littermates of the same sex and the same weight when possible were subjected to the different experimental conditions.

Twenty-seven animals were divided into 2 groups at the age of 24 to 27 days. One group (14 animals) received a standard diet. (See abstract by Patras, Galapeaux and Templeton, in these Proceedings.) The other group (13 animals) received a salt deficient diet which was made by substituting starch for the 3 per cent inorganic salt mixture in the standard diet.

Twenty-six animals were thyroparathyroidectomized at the age of 24 to 27 days and divided into 2 equal groups. One group received the standard diet while the other received the salt deficient diet.

Measurement of the rate of eruption of the upper and lower incisors was begun at the time of division into groups. The measurements were made according to the method described by Schour (Physiol. Zool. **7**: 304).

The measurements made at 14 day intervals are tabulated as millimeters in table 1.

The results show that a salt deficiency started after the animals are 24 to 27 days of age has practically no effect on the rate of eruption of the incisors during the succeeding 140 days. Thyroparathyroidectomy appreciably reduced the eruption which is further reduced if such animals are restricted to a salt deficient diet.

TABLE 1

TREATMENT	DIET	INCISORS	MEASUREMENTS IN MM. AT 14 DAY INTERVALS									
			14	28	42	56	70	84	98	112	126	140
Unoperated	Standard	Upper	3.6	3.6	3.5	3.2	3.2	2.8	3.0	3.1	3.0	3.0
Unoperated	Salt deficient	Upper	3.7	3.5	3.6	3.1	2.9	3.4	3.6	3.0	3.2	3.3
Operated	Standard	Upper	2.3	2.1	2.1	1.7	1.9	2.2				
Operated	Salt deficient	Upper	2.1	1.7	1.3	0.9	1.7	1.5				
Unoperated	Standard	Lower	6.5	5.4	5.6	5.4	5.0	5.0	4.3	4.6	5.0	5.1
Unoperated	Salt deficient	Lower	5.9	6.2	6.5	6.5	5.8	5.4	5.6	5.7	5.4	5.1
Operated	Standard	Lower	3.8	3.7	3.7	3.3	3.3	3.0				
Operated	Salt deficient	Lower	3.3	3.1	2.2	2.0	1.9	2.1				

Studies on the sympathetic nervous control of the anterior hypophysis. H. B. FRIEDGOOD (by invitation) and W. B. CANNON. Department of Physiology in the Harvard Medical School.

A previous report stated that bilateral stimulation of the cervical sympathetics of the rabbit resulted in an increased rate of secretion of anterior hypophyseal gonadotropic substances as indicated mainly by ovum maturation and occasionally by ovulation of a limited number of ova. Stimulation was carried out through a Harvard coil with 8 volts in the primary circuit, and a mechanical interrupter delivered the shocks at 25 per second. Quantitatively, the response of the anterior hypophysis to cervical sympathetic stimulation was similar to that evoked by injections of subovulatory doses of gonadotropic substance. In order to eliminate one possible cause of inadequate hypophyseal response, these experiments were repeated with another method of stimulation, viz., a condenser discharge as the source of stimulation. Ovum maturation occurred in the majority of rabbits, but ovulation was not encountered. Adequate controls were entirely negative. Because of the functional changes which occur in the anterior hypophysis after gonadectomy or thyroidectomy, unilateral gonadectomy or bilateral thyroidectomy were performed and the cervical sympathetics were stimulated several weeks thereafter. Extensive maturation of ova without ovulation occurred in the thyroidectomized rabbits; the gonadectomized animals showed similar but slight changes.

It is concluded that stimulation of the cervical sympathetics can increase to a limited extent the normal rate of secretion of the anterior hypophyseal gonadotropic substances. This evidence indirectly supports the current belief that there is another and more important neural pathway to the anterior hypophysis.

The excretion of gonadotropic substance by the normal man after administration of extracts of pregnancy urine. MAURICE H. FRIEDMAN and GEORGE WEINSTEIN (by invitation). Department of Physiology, University of Pennsylvania Medical School, Philadelphia.

Examination of the benzoic acid preparations (Katzman-Doisy method) of 24 hour urine samples of normal adult males revealed a daily excretion of an average 5 rat units of gonadotropin when assayed by the rabbit method. Tannic acid extractions (method of Louis Levin) of the same urines consistently recovered two to three times as much of the active material. The oral ingestion of 5,000 to 10,000 rat units of a pregnancy urine extract did not result in any significant increase in the 24 hour excretion of gonadotropin. However, the intramuscular injection of 500 rat units of p. u. extract was similarly without effect on the urinary content of gonadotropic substance. Only after the injection of more than 650 r. u. of the extract was there any increase in the urinary excretion of gonadotropin.

The effect of adrenalectomy upon the response of the rat to the ketogenic principle of the anterior pituitary. EDITH G. FRY (introduced by C. N. H. Long). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

The increased ketonuria developed by normal, fasted rats in response to injections of alkaline extracts of the anterior pituitary was greatly reduced by adrenalectomy, whether the animals were maintained after operation on physiological saline or on active extracts of the adrenal cortex. However, if the presence of accessory cortical tissue was either identified microscopically or indicated by long survival of the rat after withdrawal of saline or cortical extract therapy, increased ketonuria resulted from the injection of anterior pituitary extracts.

Preliminary experiments indicate that rats respond to the same anterior pituitary extracts as well following thyroidectomy as before operation.

The influence of age on the resistance to insufficient intake of inorganic salt mixture. E. A. GALAPEAUX (by invitation), E. L. BORKON (by invitation) and R. D. TEMPLETON. Department of Physiology, University of Chicago, and Loyola University School of Medicine.

The following results were obtained on 80 albino rats started on the experimental conditions a few hours after birth, and a second group of 24 rats started at the age of 25 days. The animals used were taken from our own colonies.

Within 24 hours after birth 7 litters, a total of 40 rats, were placed on a standard diet. (See abstract by Patras, Galapeaux, and Templeton in these Proceedings.) At the same time an equal number of rats were started on a salt deficient diet made by substituting starch for the 3 per cent inorganic salts.

In the second group of animals littermates of the same sex and weight when possible were used. Twelve animals received the standard diet while 12 received the salt deficient diet.

The survival record of the first group shows that by the hundredth day only 47 per cent of those on the deficient diet were living while 85 per cent of those on the standard diet survived (table 1).

In table 2 the average weights of the surviving animals are tabulated. The animals subjected to the salt deficient diet shortly after birth showed at all times a greatly retarded growth, manifest not only in the weight curve but also in the stature development. The animals started on the salt deficiency at the age of 25 days show retarded weight curve but not to the same extent as when the deficiency is started at birth.

TABLE 1

DIETS	DAYS											AGE AT START	PER CENT SURVIVAL
	0	10	20	30	40	50	60	70	80	90	100		
Salt deficient	40	36	33	32	30	26	21	21	21	21	19	Birth	47.5
Standard	40	38	35	34	34	34	34	34	34	34	34	Birth	85

TABLE 2

DIETS	NUMBER OF RATS	AGE AT START	WEIGHTS IN GRAMS ON THE VARIOUS DAYS									
			0	10	20	30	40	53	61	76	88	100
Standard	40	Birth	6	20	39	65	103	149	170	200	214	235
Salt deficient	40	Birth	6	15	24	32	42	49	59	69	72	75
Standard	12	25 days	54	123	140	146	152	174	190	211	227	244
Salt deficient	12	25 days	54	68	83	99	114	134	144	160	172	181

The effect of counterirritation upon pain produced by cutaneous injury. G. D. GAMMON (by invitation), I. STARR, JR. and D. W. BRONK. Johnson Foundation, University of Pennsylvania.

In order to study the effects of counterirritation upon pain, the skin was injured by means of irritants such as capsicum and turpentine so that the response to physical agents could be determined. Heat, electrical stimulation and rubbing exaggerated, while cold relieved the pain. The part which circulatory changes play in these phenomena was investigated by alteration of the blood supply in various ways. Obstruction of the arteries to the part promptly exaggerated, and release promptly abolished the pain; venous obstruction gradually increased the pain even for a time after the circulation was reëstablished. General vasodilatation increased the pain. The effect of temperature on the pain is not necessarily dependent on vascular change since application of heat to skin in which the vessels had been maximally dilated by means of mecholyl, histamine or heat still increased pain, as it did in skin in which adrenaline cataphoresis had caused extreme vasoconstriction. Furthermore, cold still relieved pain under conditions in which vascular changes were minimal or absent. From these observations it would appear that modification of pain by circulatory change depends on alteration of temperature, the effect of the blood flow on local concentration of the products of injury which set up pain impulses, and tissue swelling.

A study of these phenomena in cutaneous nerves in cats from similarly injured skin areas has been made. Under these conditions there is a steady discharge of rapid impulses which is greatly increased by heat with little decline in frequency due to adaptation. The question whether slow impulses are also concerned is under investigation as well as the relation of this discharge to the activity of temperature receptors.

The results of these studies provide a more rational approach for the utilization of counterirritation on skin pain.

The nervous secretion of saliva. Quantitative studies in the natural unconditioned reflex secretion of saliva. W. HORSLEY GANTT. Conditional Reflex Laboratory of the Phipps Clinic, Johns Hopkins University.

In order to evaluate fluctuations in the conditioned reflex, the laws governing the underlying unconditioned reflex should be known. The present investigation was carried out under normal physiological conditions, in the intact animal. The results show: 1. There is only slight variation in the unconditioned reflex in the laboratory life of our dogs as measured in the individual animal over a period of five years. 2. The unconditioned reflex secretion of saliva varies as a fractional exponential function of the amount of food. 3. The length of time required to eat the food has an influence on the unconditioned secretion. 4. The conditioned reflex is nearly parallel to the unconditioned reflex.

The parotid salivary secretion thus differs from the secretion of other digestive glands such as the stomach and the pancreas, where the secretion varies in a direct proportion to the amount of food.

Potentials in mammalian A fibers. HERBERT S. GASSER. Cornell University Medical College and The Rockefeller Institute for Medical Research. (Read by title.)

The duration of the spike as seen in single threshold *A* fibers (at 37°C.) is 0.4 msec., of which one-third is in the phase of rising potential. Following the spike there is negative after-potential, identifiable as such because of the associated supernormality, lasting about 10 to 12 msec.; the latter is followed by a sharply defined positive potential which reaches its greatest depth around 15 to 18 msec. and declines to zero at 60 msec. or longer.

Through the sharp positive after-potential mammalian *A* fibers are placed in contrast with frog *A* fibers, in which the positive after-potential is small or even absent after single responses, and brought more into line with *C* fibers of both forms. Compared with *C* fibers, the positive after-potential is much smaller, however. The negative after-potential, on the other hand, even under the best conditions of isolation, is larger, although the capacity for developing negative after-potential is not as large. Mammalian *C* fibers, like frog *C* fibers, when veratrinized can produce a negative after-potential as large as the recorded height of the spike.

Short tetani cause summation of the positive after-potentials. In a train of responses at constant intervals, the potential at the end of each successive interval is more positive (or less negative) than at the end of the preceding one; when the train is broken, a positivity ensues which is much deeper than the one occurring in a single response. At the same time the duration of the after-negativity is curtailed. Summation of positivity is most marked at the start of the train and tends to reach a steady state characterized mostly by the frequency of stimulation. Pairs of stimuli bring out very well the effect of frequency of stimulation upon rapidity of development of positivity. Greatest and most prolonged positivity is evoked when the second response comes soon after the relatively refractory period of the first. As the shocks are separated, the positivity from the double response decreases, but summation is still very definite at intervals of 60 msec.

In tetanized *isolated* nerves, negative potential again appears at the end of the sharp positive potential.

On the rôle of CO_2 in counteracting the effects of anoxemia on brain stem and cortex. E. GELLHORN. Department of Physiology, University of Illinois, College of Medicine, Chicago.

Air-N_2 mixtures containing 8 to 9 per cent O_2 with and without 3 per cent CO_2 were inhaled for 8 to 12 minutes and the effects on vision and muscular coördination were studied. The considerable loss in brightness distinction observed in O_2-lack (Masson discs) was either completely prevented or greatly diminished in the presence of 3 per cent CO_2. Severe disturbances in writing under O_2-lack were also absent when 3 per cent CO_2 was simultaneously inhaled. Whereas the number of nystagmic movements resulting from galvanic stimulation of the vestibular apparatus was greatly diminished under O_2-lack (6.5 per cent) and led not infrequently to a complete absence of response, nystagmus remained unaltered, i.e., the number of responses was the same as in air when the rabbit inhaled 6.5 per cent O_2 + 5 per cent CO_2. The explanation of these results is probably to be found in the improvement in respiration and consequently in circulation due to CO_2 as well as in the changes of the O_2-dissociation curve. The circulatory changes are illustrated by experiments in the human in which it was found that under the influence of 8 to 9 per cent O_2 in the erect position the systolic blood pressure dropped considerably after an initial rise, thereby leading eventually to collapse. The presence of 3 per cent CO_2 was sufficient to maintain a normal blood pressure under the same conditions. The relevant literature (Mosso, Margaria, Henderson and collaborators) is discussed.

The respiratory metabolism of stimulated frog's muscle. C. L. GEMMILL. Johns Hopkins University, School of Medicine, Baltimore, Md.

The respiratory metabolism of the stimulated frog's sartorious muscle was determined by a modification of the Dixon-Keilin method. The respiratory quotient averaged 0.90 with variations from 0.86 to 0.98 in the bicarbonate buffered solutions. The oxygen consumed per gram of muscle per hour averaged 357 mm.^{3} and the tension developed gave an average value of 195 kgm. per gram of muscle per hour.

A direct proportion was found between the oxygen consumed and the tension developed in thirty-five experiments on the frog's sartorius stimulated under aerobic conditions.

The effect of yeast on the survival period of albino rats in experimental hyperthyroidism. VALERIA GENITIS (by invitation), JANET TOWNE (by invitation), MARY C. PATRAS (by invitation) and R. D. TEMPLETON. Department of Physiology, Loyola University School of Medicine.

A summary of results obtained on 162 albino rats showing the relative value of Baker's and dried brewer's yeast in experimental hyperthyroidism is presented. The animals used were taken from our own colony which receives a stock diet of Fox Chow ad libitum, and meat, bread and cabbage once weekly.

Littermates of the same sex and weight, when possible, ranging from 177 to 288 days were divided into 6 groups. Group A received a standard diet. (See abstract by Patras, Galapeaux and Templeton in these proceedings) Groups B and C received the standard diet modified by the addition of 0.05 per cent and 0.1 per cent desiccated thyroid respectively.

In order to increase the yeast concentration for group D, without materially effecting the quantity of the other dietary constituents in the standard diet, 10 per cent dried brewer's yeast (Northwestern Yeast Co.) and 5 per cent starch were substituted for the 15 per cent baker's yeast

(Fleischman's). This diet was further modified by addition of 0.05 per cent and 0.1 per cent desiccated thyroid for groups E and F respectively.

The results presented in the table show practically no difference in the survival period of the animals receiving the control diets (groups A and D).

GROUP	PER CENT YEAST	PER CENT THYROID	NUMBER OF ANIMALS	200 DAYS		400 DAYS	
				Number living	Per cent living	Number living	Per cent living
A	15 Baker's	0	32	31	96.8	27	84.3
B	15 Baker's	0.05	22	17	77.2	6	27.2
C	15 Baker's	0.1	34	7	20.5	2	5.9
D	10 Brewer's	0	27	26	96.3	22	81.5
E	10 Brewer's	0.05	16	13	81.2	8	50.0
F	10 Brewer's	0.1	31	13	41.9	2	6.4

After 200 days on the diets containing thyroid a beneficial effect was noted where dried yeast was used. Where only 0.05 per cent thyroid was used in the diet a similar beneficial effect was noted 400 days after the beginning of the experiment. Where 0.1 per cent thyroid was used the beneficial effect seen in the first 200 days was not carried to the 400th day.

Localized thermal changes in brain. R. W. GERARD and H. SEROTA (by invitation). Department of Physiology, University of Chicago.

The Horsley-Clark instrument has been used in this laboratory to localize electrical activity and oxygen consumption in particular brain tracts and centers. Similar studies of local temperature changes are here reported. A needle thermojunction was placed by the instrument in the optic tract, lateral geniculate body or optic cortex, or in the cutaneous radiations of a cat, and current variations followed with a sensitive Zernicke galvanometer (1 mm. = 0.00075 C.). On illuminating the eyes (up to a minute), with the needle in the optic pathways a temperature rise begins within a minute and increases for about two more, the original baseline being regained in another four. The average increase is roughly 0.01°C. Pinching, stroking or massaging the feet is without effect for these needle positions but leads to similar thermal changes when the thermocouple is in the cutaneous paths. At some points both are effective, and action potential responses to both types of stimuli have also been obtained at a single setting. In three experiments, recording from the geniculate, light led to a temperature fall rather than rise.

Clearly vasomotor effects are involved in these changes, although increased heat production by active neurones may also contribute. Extensive controls of the effect of asphyxia, carotid occlusion, anesthetics, etc., on brain temperature, as well as measurements in various blood vessels, indicate that the needle may function as in either the Gibbs or the Schmitt method for blood flow measurement, depending on body and outside temperature relations.

A general brain vasodilatation results from O_2-lack and CO_2-excess; a highly localized one occurs in active brain regions—probably on a similar chemical basis. The above results were obtained alike under ether or nembutal anesthesia, although the former leads to a marked dilation (0.05° increase in two minutes after a strong whiff), the latter rather to a long constriction.

Fusillade patterns of inspiratory and expiratory muscles and their mechanical effects on the respiratory act. ROBERT GESELL. Department of Physiology, University of Michigan.

Fusillade patterns of inspiratory and expiratory contractions during eupnea were compared and their mechanical effects considered.

Inspiratory contractions were characterized by a *progressively accelerating* rate of muscle fiber twitching and by a *progressive recruitment* of fibers up to the end of inspiration. Expiration was initiated by a *sudden* reversal of this inspiratory innervation. This mode of innervation and denervation produced a triangular configuration of the electrogram indicating the changing strength of muscular contraction.

Expirations were of three types: 1, passive; 2, "rectangular"; 3, "triangular." Rectangular expiration was characterized by a uniform muscle fiber rhythm and uniform number of action potentials. Triangular expiration was characterized by a maximum number of potentials at the *beginning* of expiration *progressively diminishing* with time.

It is suggested that rectangular and triangular expirations may be fundamentally different: that triangular expiration is a product of a direct discharge from the expiratory side of the respiratory mechanism; that rectangular expiration is an after effect of inhibition of a tonic viscero-postural reflex.

This conception is supported by experiments on the transcostar muscle. Reflex contraction elicited by weighting the free end was rhythmically interrupted with each inspiration giving all indications of active expiratory contractions, which they may be.

A sporadic deep breath which enormously augments the inspiratory fusillade may have no effect upon the succeeding expiratory fusillade, though the expiratory excursion is correspondingly increased. This shows that inspiratory and expiratory innervations may be separately modified supporting paragraph 4.

It is proposed that proprioceptive reflexes contribute towards the guidance of the respiratory act by influencing the incidence, strength and sequence of contraction of respiratory muscles.

Mechanically, waxing inspiration meets increasing resistance producing a smoothly conducted inspiratory act, but the sudden change in phase of respiration at the height of inspiratory discharge produces the characteristically uneven filling of the lungs.

The sudden liberation of expiratory forces at the beginning of expiration favors jerky expirations but waning mechanical forces meeting increasing resistance to deflation produces the characteristically even emptying of the lungs.

The effects of various stimuli on the shifting of blood in experimental animals. CESARE GIANTURCO (by invitation) and F. R. STEGGERDA. Carle Hospital Clinic and the Dept. of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

The visualization of the circulatory system to x-rays by replacing approximately one-fourth of the blood volume with Thorium Dioxide Sol (Thorotrast) affords a convenient method for studying changes in size of the larger arteries and veins in the entire animal (rabbit) for nearly one hour. One hour after the injection, the medium disappears from the circulatory system and accumulates in the liver and spleen.

The effects of such stimuli as histamine, ephedrin, adrenalin, air embolism, oil embolism, fracture of the skull, and pituitrin were studied, although only one of these can be reported here. A lethal dose of histamine decreases the volume of the systemic and pulmonary arteries, dilates the veins of the systemic circulation, congests the liver, reduces the size of the left ventricle, and dilates the right ventricle. The other stimuli also showed characteristic effects.

Although this method has its limitations, it has the advantage that all the visible vessels of the body can be examined at the same time.

Localizable features of the electrical activity of the brain in petit mal epilepsy. F. A. GIBBS, W. G. LENNOX (by invitation) and E. L. GIBBS (by invitation). Department of Neurology, Harvard Medical School.

The three-a-second wave and spike which was described as characteristic of petit mal epilepsy has now been seen in 34 patients with this disorder, in no normal subjects and in no patients suffering from other disorders. The spike is more evident if the active electrode is placed over the frontal region. With such a lead, the slow component has a dome-shaped top. Over the two motor areas the potential fluctuation is not so great, and the slow wave tends to have a peaked top. From the high occipital region a large irregular pyramid shaped wave is obtained and the spike appears only occasionally. Activity tends to be synchronous over homologous areas on the two sides. The chief focus of this pathological electrical activity in petit mal epilepsy appears to lie in the frontal area.

The effect of diminution of barometric pressure on the sensitivity of rats to insulin. N. GLICKMAN (introduced by E. Gellhorn). Department of Physiology, University of Illinois College of Medicine, Chicago.

In order to further test the anoxemia theory of insulin convulsions (cf. Olmsted and collab. 1923/24 and Dameshek and Myerson 1935), the reaction of rats to insulin under normal and low barometric pressure (460 mm.) was studied. The insulin test under low pressure was preceded and followed by a control test under normal pressure. The results of 43 experiments were: in 36 experiments (84 per cent) the onset of convulsions was enhanced under low pressure; no effect was observed in 3 cases (7 per cent), and it was delayed in 4 experiments (9 per cent). The conclusion drawn from these experiments that insulin becomes more effective under low barometric pressure is further shown by the fact that in 14 experiments the rats showed convulsions in the low pressure chamber, but not in control experiments. Furthermore, 17 animals which had tolerated the insulin effect under control conditions died in the low pressure chamber. The low barometric pressure itself was, as control experiments showed, without any effect on the animals during the period of observation.

A study of ketosis in primates. WALTER GOLDFARB (introduced by H. E. Himwich). Laboratory of Physiology, Yale University School of Medicine, New Haven.

Experimental work reported by Shaffer, Woodyatt and others indicates that the oxidation of ketogenic substances is catalyzed by the simultaneous oxidation of antiketogenic substances. These results have been confirmed by us in observations on white rats. In the present study the investigation was extended to include primates. Eight experiments on 7 monkeys are

reported. The animals were fasted and then injected with 100 mgm. of phlorhizin daily. The foodstuffs metabolized were determined from analyses of metabolic rats, R.Q., urinary nitrogen, and acetone substances for periods of 24 hours. Assuming a ketogenic:antiketogenic ratio of 2:1, the amounts of acetone substances that should be excreted were calculated and compared with the observed recovery in the urine. The theoretical yields exceeded the observed yields 6 times, and were less twice. These discrepancies could be counted for by errors inherent in the method of determining the respiratory quotient. An human patient yielded results which closely approximated those of the present study. In view of the similarities in the experiments on the white rat, monkey and human, it seems highly probable that the complete oxidation of ketogenic substances in the mammalian organism requires the simultaneous oxidation of definite proportions of antiketogenic equivalents.

Phenol red, inulin and urea clearances in renal disease. WILLIAM GOLDRING (by invitation), ROBERT W. CLARKE (by invitation) and HOMER W. SMITH. Department of Medicine, Third (New York University) Division, Bellevue Hospital, and the Department of Physiology, New York University College of Medicine.

In view of the large order of magnitude of the whole blood phenol-red clearance in normal man, (J. Clin. Invest., March, 1936) and the possibility of measuring the effective blood flow through the normal human kidneys by the clearance method, it was deemed important to determine to what extent the renal secretory activity could be impaired by disease. Two series of individuals, one having characteristic signs of essential hypertension, the other of chronic diffuse glomerular nephritis, have been examined in respect to phenol red, inulin and urea clearances. It appears that in general the phenol red and inulin clearances are affected in a similar manner, though the phenol red clearance is depressed to a relatively greater extent. In advanced renal disease the phenol-red/inulin clearance ration may be under 1.00 (in contradistinction to the normal value of 3.0 or greater), indicating that while renal tissue may still be functional with respect to filtration, the secretory capacity may be so impaired that the phenol-red clearance approaches the filtration clearance of the dye, which, in view of the small fraction of filterable dye in the plasma, would be a corresponding fraction of the inulin clearance.

The urea clearance is likewise affected in a parallel manner with the inulin clearance, but contrary to phenol red, the clearance of which is depressed to a greater extent than that of inulin, the urea clearance is depressed to a lesser extent than is the inulin clearance. At urine flows above 1.0 cc. per minute the urea/inulin clearance ratio rises from its value of 0.64, as observed in the normal individual, to 0.80 or higher. In view of the large molecular weight of inulin and of its relatively low diffusibility, this implies a diminished (rather than an increased) reabsorption of urea in the types of pathological kidney which we have examined.

The metabolism of bilaterally nephrectomized dogs. J. L. GOUAUX (by invitation), SHIRLEY C. CORDILL (by invitation) and A. G. EATON. Department of Physiology, Louisiana State University Medical Center, New Orleans, Louisiana.

Studies were made of basal metabolism and blood urea nitrogen on two series of dogs anesthetized with sodium amytal. In both series, control

experiments consisted of three basal metabolic determinations; three determinations of blood urea, the first taken before the anesthetic, the second approximately 90 minutes later and the third three hours after the sodium amytal respectively; and a determination of urine urea nitrogen for the three hour control period. Bilateral nephrectomy was performed with as little trauma as possible. In series I fourteen dogs were used and their metabolism was followed for five hours after the operation. Series II consisted of twenty-one animals, most of which were used for a study of the specific dynamic action of glycine. Their metabolism was followed for two hours after nephrectomy. Five dogs, operated upon in exactly the same way, except that the kidneys were not removed, served to measure the effect of trauma.

Although there was considerable individual variation, the heat production fell in all cases showing an average decrease of 15.2 per cent at the end of two hours in series I, 12.7 per cent in series II and 13.1 per cent in series I for the five hour period. The controls showed a decrease of 8.9 per cent for the two hour period and 5.8 per cent for the five hour period. Thus the actual decrease in heat production due to bilateral nephrectomy amounts to from 4 to 7 per cent of the total metabolism. Calculated on the basis of net decrease per gram of kidney per hour in series I, the values are 0.026 Cal. for two hours, 0.029 Cal. for five hours. For series II (for two hours) the decrease is 0.017 Cal. The R. Q. increased from 0.751 to 0.805 for the five hour series, but was not changed significantly in either the control or the two hour experiments. Blood urea nitrogen practically doubled in five hours (13.9 to 26.1 mgm. per cent.)

Rate of conduction in mammalian nerves in vivo. HELEN TREDWAY GRAHAM and R. LORENTE DE NÓ (by invitation). Dept. of Pharmacology, Washington University Medical School, St. Louis, Missouri and Central Institute for the Deaf, St. Louis, Missouri.

The interval between the application of electrical shocks to the sciatic nerve and the appearance of the response in the peroneal nerve at varying distances from the point of stimulation, has been measured in rabbits under conditions approximating normal as closely as possible. The cathode ray oscillograph used permits the making of photographs at spot speeds of 500 m./sec., and the timing apparatus is accurate within 10 microseconds or less. From the data obtained are drawn the following conclusions regarding the rate of conduction of the nerve impulse. 1. The average rate of conduction through the nerve rarely exceeds 100 m./sec. and is usually somewhat less than this. 2. In either decorticated or etherized rabbits the conduction rate does not vary from moment to moment, and remains quite constant for several hours. 3. Severing the nerve some distance above the point of stimulation has never been found to increase the rate of conduction. 4. During the supernormal and subnormal periods with a degree of excitability as high as 12 per cent above or as low as 33 per cent below the resting level, no change in the conduction rate has been found greater than the limit of accuracy of the measurement of the records (1 to 2 per cent).

Cardiovascular changes resulting from stimulation of the cerebral cortex. HAROLD D. GREEN (by invitation) and EBBE C. HOFF. Laboratory of Physiology, Yale University, New Haven.

In acute experiments carried out under ether anesthesia on 35 cats and

12 macaques (*M. mulatta*) curarized to abolish somatic muscular responses, the cerebral cortex was explored with bipolar electrodes activated either by a thyratron stimulator or a Harvard inductorium. Records of blood pressure, heart-rate, and the kidney and limb volumes were obtained either by mechanical or optical registration. From the gyrus proreus and the sigmoid gyri in cats and the cortex adjacent to the superior precentral sulcus (areas 6 and 4) in monkeys, rise of blood pressure, acceleration of heart-rate, reduction of kidney volume, and increase in the volume of both hind legs were regularly obtained. Decline of pressure frequently resulted from stimulation of the lateral, suprasylvian and ectosylvian gyri in cats.

In 3 cats and 1 monkey bilateral stellate ganglionectomy and adrenalectomy reduced but did not abolish the augmentation of blood pressure and limb volume resulting from cortical stimulation. Secondary rises of pressure frequently occurred several seconds after stimulation. Each rise and fall of blood pressure was accompanied by a corresponding dilatation and contraction of the pupils, and a retraction and relaxation of the nictitating membranes. Removal of one stellate ganglion eliminated the responses of the pupil and nictitating membrane on the same side. In one monkey the hair over the shoulders was repeatedly elevated by excitation of the cingulate gyrus.

The following evidence indicates that these responses are initiated by cortical elements: 1, nupercaine applied to a small area of the cortex gradually abolished blood pressure responses from this point, but the rise in pressure was reobtained by inserting the electrodes 3 to 5 mm. into the brain; 2, undercutting the gray matter abolished the responses; 3, the changes in pressure were elicited by stimuli just strong enough to evoke contraction of skeletal muscles when applied to the cortex of non-curarized animals; 4, "rise" and "fall" points could be localized within 2 to 4 mm. of one another. Under sodium amytal anesthesia vaso-pressor responses could not be obtained from the cortex.

The coördirectograph. HAROLD D. GREEN (introduced by C. J. Wiggers). Western Reserve University, School of Medicine. (Demonstration.)

The coördirectograph (demonstrated) is a mechanical device designed to enlarge or reduce proportionally any plane geometric curve in one direction without altering its dimensions in the direction at right angles to the first. It is described in the Rev. Sci. Instr. **7:** 1936.

The device was originally constructed to facilitate the quantitative comparison of two pressure curves recorded simultaneously by optical manometers of unequal sensitivity, the instrument being used to expand or reduce the ordinate dimensions of one of the curves until it had the same scale value as the other (in terms of mm. Hg pressure per millimeter distance).

Other suggested physiological uses for the device are: to aid comparison of myographic curves recorded under different conditions; to facilitate comparison of oscillograph tracings recorded under conditions of different sensitivity and to allow comparison of curves recorded on drums which have not been operated at the same speed.

The contrivance may also be used to condense or expand single curves to accentuate their inflections; it may be used to subtract quantitatively curves with equal or unequal ordinate scales, and also provides a convenient

means for constructing an exact ellipse from a circle of the same radius as the semi-major or semi-minor axis of the ellipse.

The machine is capable of enlarging curves 1.25 to 5 times their original size and of reducing them 0.8 to 0.2 times their original size.

Quantitative determination of lactic acid in body fluids by means of iodine pentoxide. LEON A. GREENBERG. Laboratory of Applied Physiology, Yale University.

The iodine pentoxide train affords an accurate and rapid means for the quantitative determination of all combustible volatile organic substances. When lactic acid is heated with potassium permanganate or with a suspension of manganese dioxide it is oxidized to carbon dioxide and acetaldehyde. Since acetaldehyde can be determined accurately in small amounts by means of the iodine pentoxide train a convenient method is thus available for the estimation of lactic acid.

In determining lactic acid in body fluids by this method the removal of protein, sugars, and other interfering substances is first carried out with the customary reagents. A measured sample of the fluid is heated for a short time to remove such volatile substances as acetone, and acetoacetic acid which also react with iodine pentoxide. This sample is then diluted with water in a flask with an air inlet and connected to a reflux condenser. The effluent end of this condenser is connected to one end of the U-tube containing alternate layers of iodine pentoxide and glass wool and maintained at a temperature of 160°C. A steady stream of air is drawn through the train by suction.

Solutions of manganous sulphate and phosphoric acid are added in succession to the flask containing the fluid under analysis, which is then heated to boiling. All of the lactic acid in the fluid is thus oxidized to carbon dioxide and acetaldehyde. The acetaldehyde is drawn through the hot iodine pentoxide and, if the tube is fresh, it liberates free iodine quantitatively; and the iodine is collected by bubbling through a solution of potassium iodide. The iodine is estimated by titration with a standardized solution of thiosulphate, using starch as an indicator, the amount of iodine liberated serving as a basis for calculation of the quantity of lactic acid in the original fluid. If the tube is not fresh, some of the iodine comes off as HI, but is easily collected and calculated as described by H. W. Haggard and L. A. Greenberg (J. Pharmacol. Exper. Therap. **52:** Oct. 1934).

The effect of glycocyamine upon respiration. LEON A. GREENBERG and YANDELL HENDERSON. Laboratory of Applied Physiology, Yale University.

Guanidine and some of its compounds and derivatives have marked pharmacological effects. One of these derivatives, glycocyamine, is particularly interesting because it occurs in metabolism or at least in the urine. We have conducted some experiments to determine the effects of glycocyamine upon respiration. Various doses were injected into the external jugular veins of dogs under moderate morphine or chloralose narcosis. Arterial pressure and respiration were recorded, the animal breathing through a tracheal cannula into a small spirometer filled with oxygen and containing soda lime.

With doses of glycocyamine as small as 5 mgm. per kilo the effects were

distinct. Arterial pressure, after a brief rise, dropped below its normal level and then slowly returned. Respiration was immediately and markedly increased both in volume and frequency, but gradually subsided to its normal value again in 4 or 5 minutes. Larger doses of glycocyamine produced changes of the same type but lasting longer until toxic doses (30 mgm. per kilo) were reached, when the animal died of respiratory failure with typical asphyxial gasps.

Observations on the expiratory chest volume during air hunger produced by physical exertion. JAS. A. GREENE and L. W. SWANSON (introduced by F. M. Smith). Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City, Iowa.

The expiratory chest volume of man was measured following physical exertion to ascertain whether or not the volume of air in the lungs at end of expiration decreased or increased during air hunger thus produced. In previous studies a decrease of expiratory chest volume was not observed, whereas inconstant increases occurred during measurement of basal oxygen consumption and during rebreathing experiments in which there was oxygen deficiency or excess of carbon dioxide or both.

The changes in chest volume were recorded on a kymograph by means of a plethysmographic bag previously described. The subjects reclined in a chair and pedaled a stationary bicycle. Since activity disturbed the plethysmographic bag, observations were made during the recovery period following physical exertion. Each subject continued the exertion until he became definitely short of breath. The theoretical distance the bicycle traveled and the duration of exertion were noted. The pulse rate and blood pressure were recorded before and at one minute intervals after the exercise.

Thirteen observations were obtained on nine normal subjects and four observations on three subjects with arthritis of the spine. The results show that the expiratory volume of the chest increases during air hunger produced by moderate physical exercise. This increase occurs even in subjects in whom excursions of the thoracic cage is prevented by arthritis of the spine.

Flexion of the spine, the degree of relaxation of the subject, and possible errors inherent in the apparatus have been excluded as sources of errors in the above observations.

The effect of adrenalin on the plasma volume of normal and sympathectomized dogs. MAGNUS I. GREGERSEN and JAMES O. PINKSTON (by invitation). Department of Physiology, Harvard Medical School.

A total of 35 experiments were done on 8 normal and 9 sympathectomized dogs, trained to lie quietly during the observations. Adrenalin (1/5000 or 1/2000) was given intravenously at a constant rate of 0.005 to 0.006 mgm. per kilogram body weight per minute for 30 to 40 minutes. The total volume of solution injected in each experiment never exceeded 10 cc.

The plasma volume was determined with the blue dye T-1824 and spectrophotometric analysis of serum samples (Gregersen, Gibson and Stead, 1935). Further evidence of volume changes was obtained from determinations of serum proteins (refractometer).

Contrary to the impression to be had from the literature (Freeman, 1933), adrenalin does not invariably cause a fall in plasma volume. In at

least $\frac{1}{3}$ of our experiments on normal dogs, we found either a rise (5–10 per cent) or no definite change in volume. In the remaining experiments, there was a fall of from 5 to 15 per cent. The adrenalin effect, when it occurs, takes place within 5 to 10 minutes, and continuance of the injection beyond this point appears to accomplish nothing more than to maintain the new volume level.

All the sympathectomized dogs responded to adrenalin with a decrease in plasma volume. As a rule, the change was greater than in the normal dogs displaying a fall.

The average plasma volume for the normal dogs was 46.6 cc. per kilogram body weight. The variations among the animals ranged from 37.5 to 61.5 cc. per kilogram. For the sympathectomized dogs, the average volume was 60 cc. per kilogram, with variations from 47 to 73 cc. per kilogram. Observations were made on three of these sympathectomized dogs before as well as after the operation. Their plasma volumes increase by 10, 17 and 20 per cent respectively after total sympathectomy.

The systolic and diastolic blood flow in the right coronary artery. D. E. Gregg. Western Reserve University, Medical School.

The phasic variations of blood flow in the right coronary artery have been investigated by the method of differential pressure curves which has already been applied to a study of blood flow in the left coronary artery (Am. J. Physiol. **112:** 362, 627, 1935). Upon the basis of the experiments to date, the P.C.P. curve (peripheral coronary pressure) and the blood flow in the right coronary artery have the following characteristics:

The P.C.P. curve starts to rise, generally during the latter part of the isometric contraction period of the right ventricle and continues to rise reaching its highest point late in systole. Then the curve declines and attains its diastolic level during the time right ventricular inflow starts. With normal right and left intraventricular pressures prevailing the diastolic coronary resistance ranges from 15 to 20 mm. Hg. The maximum coronary systolic resistance generally exceeds by a rather small amount the right intraventricular pressure.

The time relations, contour and diastolic ordinate value of the P.C.P. curve agree with those of the left coronary but the systolic resistance in the right coronary, although in excess of right intraventricular pressure, is much lower than in the left coronary artery.

Differential pressure curves indicate that the velocity of blood flow throughout most of systole, and hence the volume flow, tend to equal or exceed that during an equivalent interval of diastole. This contrasts with the flow in the left coronary in which the systolic velocity and volume flow, although considerable, are generally much lower than the diastolic.

Irritability of mammalian nerve after excitation. Harry Grundfest and Herbert S. Gasser. The Rockefeller Institute for Medical Research.

The generalized form of the recovery curve of nerve fibers is one which may be described as follows. After a period of absolute refractoriness, irritability rises rapidly to a maximum. Then there follows a period of gradual decline, which is succeeded by a slow return to the resting level.

The generalized form manifests variations, depending upon the type of fiber, the condition of the nerve, and the extent of the activity. The initial maximum may occur at normal, supernormal, or subnormal irrita-

bility; and the subsequent decline may be small or very marked. Mammalian *A* fibers display all of these variations.

Certain isolated nerves in states of high irritability have a greatly exaggerated supernormal phase, the threshold dropping to one-half after 4 msec. Decline of irritability to normal occurs within about 15 msec. (potential negative) and progresses into a period of subnormality lasting throughout the positive potential. In such nerves, a single maximal response interpolated in a threshold tetanus, at rates above 100 per second, brings the tetanus to maximal; and the tetanus is so continued for a time because the stimuli fall in the supernormal phases of the preceding responses.

Nerves in the body, functioning within the range of conditions which may be classed as physiological, also evidence supernormality, but to a less degree Unexposed nerves with their natural circulation were tested in decerebrated cats at points other than the locus of the conditioning shocks, and the result of the stimulation was recorded electrically from a distant, exposed portion of the nerve. Maximal irritability occurred at 6 to 8 msec. (supernormality 2 to 15 per cent), and maximal subnormality (about 4 per cent) at 25 to 35 msec. Normal was reached after 50 to 80 msec.

Conditioning with a tetanus depressed the irritability throughout the course of the curve of recovery, without changing its general form. A family of curves, showing increasing degrees of subnormality, was obtained as the frequency and duration of the conditioning tetanus was increased, the former variable being more important than the latter. The curves with subnormal initial maxima obtained after short tetani resembled the recovery curves of *C* fibers as they occur after single responses.

Uterine temperature during oestrus, pregnancy and other physiological changes in the unanesthetized rat. JAMES B. HAMILTON (introduced by H. E. Himwich). Departments of Anatomy, Yale Medical School, and Physiology, Albany Medical College.

By previously implanted thermocouples, uterine temperature was measured during normal conditions, physiological changes (oestrual rhythms and pregnancy), hypothermia and hyperthermia.

Results. Physiological changes in the uterus. Concomitant measurements of uterine and body (colonic) temperatures revealed that the uterus conformed to the level of body heat during all stages of the oestrual cycle in the rat. This agreement of organic and body temperatures persisted undisturbed by gestation, postpartum conditions of lactation and involution or even the severe muscular disturbances of parturition. Neither the injection of theelin in doses large enough to cause marked uterine growth nor ovariectomy with subsequent castration atrophy affected the uniformity of temperatures. Epinephrine and pituitary extracts were used to produce rapid response, but no change in uterine temperature was observed save when the body temperature exhibited a like and concomitant change.

Changes in general body temperature. In a cold environment the body temperature of a rat can be reduced without the aid of anaesthesia as much as 45°F., below its normal level with subsequent spontaneous recovery. In all stages of this extreme drop the uterine and body temperatures continued in unison, regardless of whether the organ was in a highly vascular

state as in oestrus or pregnancy, or whether it was atrophied as after ovariectomy. In environmentally-produced hyperthermia this similarity of the organic and body degree of heat also persisted, even though the body heat level attained and exceeded the lethal point. Stimulation of the body by drugs to produce a febrile condition and the converse, a drug-produced loss of heat, likewise gave a similar uterine-body degree of heat.

Summary. The uterus of the unanaesthetized rat was studied during physiological changes which included all stages of the oestrual rhythm, pregnancy, parturition, lactation, involution, rapid uterine growth, and castration. Uterine temperature was also observed at body heat levels ranging from 55° to 110°F. The temperature of the uterus was found to conform to the body (colonic) level of heat at all times. These results give no evidence for the many claims of temperature differences among the abdominal organs.

Simultaneous changes in human intrathoracic and arterial pressure during respiration, coughing and straining. W. F. Hamilton, R. A. Woodbury (by invitation) and H. T. Harper (by invitation). Department of Physiology and Pharmacology and the Department of Medicine, University of Georgia School of Medicine, Augusta, Georgia.

By means of the hypodermic manometer described before this group two years ago, it has been found possible to follow simultaneous changes in intrathoracic pressure and in arterial pressure. Subjects were human patients whose lungs had been collapsed for the treatment of pulmonary tuberculosis.

An increase in intrathoracic pressure (strain) dams back blood from the right heart in the venous cisterns. This causes a progressive reduction in the pulse pressure and interesting changes in the contour of the pressure pulse. At the same time the increased intrathoracic pressure acts directly to compress the vascular system within the thorax causing a rise in diastolic pressure.

A marked reduction in intrathoracic pressure (stoppage of the strain) causes a drop in both diastolic and systolic pressure followed by a rebound to higher levels after the blood dammed back in the veins has traversed the lesser circulation.

Much greater increases in intrathoracic pressure occur during coughing. These are related to simultaneous increases in the arterial pressure which may maintain a level during diastole which is twice that of the ordinary systolic figure.

Subtracting the intrathoracic pressure from the arterial gives the net intrathoracic arterial pressure (effective coronary pressure). This net pressure does not fluctuate as does the systemic blood pressure, and it continues to show the cardiac rhythm plus certain fluctuations superimposed by thoracic movements.

Postural reactions to electrical stimulation of the interior of the cerebellum in the cat and monkey. Kendrick Hare (introduced by S. W. Ranson). Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

The excitability of the interior of the cerebellum of normal monkeys and normal and decerebrated cats was demonstrated by electrical stimulation, using the Horsley-Clarke instrument. Cerebellar activation characteristically produced a biphasic response. The first phase was coincident with

stimulation; the second occurred as a rebound posture lasting, in some cases, for as long as five minutes. A second stimulus during this rebound produced an inhibition of this posture and sometimes a contraction of the antagonists of the muscles active during the rebound. This phase lasted only as long as the period of stimulus, and when it was concluded the rebound posture was immediately resumed.

Cerebellar stimulation initiated three distinct types of response: 1. That involving eyes, head, trunk and tail and all four limbs. During the first phase there was either an inhibition of any preëxisting muscular contraction, or the eyes, head and concavity of the vertebral axis were directed toward the side on which the point of stimulation was located. The ipsilateral limbs were flexed, the contralateral extended. The instant stimulation was stopped the above gave way to an opposite posture, the rebound. The concavity of the vertebral column was directed to the side opposite the cerebellar point stimulated. The ipsilateral limbs were rigidly extended; the contralateral strongly flexed. Reactions of this nature were obtained from the base of the folia of the vermis, the underlying white matter and the fastigial and globose nuclei.

2. Reactions from the emboliform nucleus and the overlying white matter, generally confined to the ipsilateral forelimb. During stimulation the limb was relaxed and on rebound was flexed.

3. Scratching and grasping, usually with only the ipsilateral forelimb, during the stimulatory phase. The rostral end of the cerebellar nuclei and the vicinity of the superior cerebellar peduncle yield this response. This was observed only in the monkey.

The efferent paths for these responses were not established beyond the fact that the brachium conjunctivum is not essential for the first two types of response. These could be obtained in the cat after decerebration caudal to the red nuclei.

The relation of pancreatic juice to pancreatic diabetes. HERMAN P. HARMS (by invitation), JOHN VAN PROHASKA (by invitation) and LESTER R. DRAGSTEDT. Department of Surgery of The University of Chicago.

The question of the relation of pancreatic juice to pancreatic diabetes has been reopened by the reports of W. Boldyreff and E. Boldyreff that the total loss of pancreatic juice in dogs produces diabetes and that the absorption of pancreatic juice from the small intestine relieves the condition. Total pancreatic fistulae were prepared in six dogs by a method previously described by one of us (L. R. D.). These animals secreted from 5 to 1400 cc. of juice daily on a standard diet. At no time did sugar appear in the urine and the blood sugar remained within normal limits. Examination of the livers of these dogs at autopsy failed to reveal fatty infiltration or degeneration. Ten dogs were completely depancreatized and kept moderately sugar free with suitable doses of insulin. They were placed on a standard diet and given from 100 to 1100 cc. of fresh activated dog pancreatic juice daily by mouth. While the nutritive state of these animals improved somewhat in no case did the pancreatic juice lessen the insulin requirement, lower the blood sugar, or reduce the sugar excretion. In fact the sugar excretion was usually increased, probably due to better absorption of glucose forming substances from the intestine.

The concentrations of adrenalin and pituitrin required to affect uterine motility, when they are administered by different methods. O. G. HARNE and E. E. PAINTER (by invitation). Department of Physiology, University of Maryland, School of Medicine.

Employing a cannulation-suspension method for recording uterine motility in the rat we have found that the application of pituitrin and adrenalin to the serous surface of the uterus excised or in situ, evokes a prompt and powerful reaction. A concentration of approximately 1–5,000 of these preparations produces what we have chosen to call a *"typical reaction."* We have used this as a basis for determining the minimum concentrations necessary to evoke the same response when the solutions are given by intravenous, intramuscular and intra-uterine injections. With intravenous injections, it was necessary to give a quantity sufficient to establish in the blood a theoretical concentration of 1–2,000, and with intramuscular injection, a theoretical concentration of 1–200. A concentration of 1–25 injected into the lumen of either the cannulated or the control horn elicited the "typical reaction." The same response is obtained with a dilution of 1–200, if the uterine mucosa is first washed with a muco-solvent (1 per cent caroid).

A study of intravascular coagulation. STEARLEY P. HARRISON (by invitation) and EDWARD C. MASON. University of Oklahoma Medical School.

Tissue extract has long been recognized as a potent coagulant substance. Recent clinical investigations have tended to place much emphasis upon this substance with respect to the problem of intravascular clots, or thrombosis. At the present time it is considered one of the vital factors present in this type of disorder.

It was found possible to produce, experimentally, intravascular clots in an artificial circulatory system. A relatively large volume of whole blood and minute amounts of tissue extract was used.

Clots removed and subjected to microscopic examination revealed a striking similarity of structure to that of the pathological *in vivo* forms.

It is our contention that, apart from the streaming action of circulating blood, intravascular and extravascular coagulation are identical.

We conclude that tissue extract should be considered the primary cause in the form of intravascular clots.

The permeability of the frog liver to certain lipoid-insoluble substances. CHARLOTTE HAYWOOD (introduced by M. H. Jacobs). Physiological Laboratory, University of Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Massachusetts.

The permeability of the isolated bull-frog liver has been investigated by adding certain lipoid-insoluble substances to the perfusion fluid and collecting the secretion for analysis. The substances added were mostly non-metabolized carbohydrates of varying molecular size: xylose, lactose, inulin and soluble starch. The addition of the dye, eriocyanin, to the perfusion fluid was routinely employed as a criterion for the survival of the liver, since the active liver is known to concentrate dyes markedly.

Perfusion pressures of 6 to 8 cm. H_2O were used in the more critical experiments. Secretion samples for analysis were collected in a cannula

tied into the neck of the gall bladder, close to the cystic ducts. Determinations for reducing sugar were made by the Shaffer-Hartmann-Somogyi micromethod, preceded when necessary by treatment of the sample with washed yeast, to distinguish between fermentable and non-fermentable sugar content. In analyses for inulin, preliminary boiling with H_2SO_4 was used.

Even when the perfusion fluid contained no reducing substance, amounts of fermentable reducing substance, in most cases less than 0.05 per cent as glucose, appeared in the secretion. The addition of 0.05 per cent xylose, 0.15 per cent lactose, and 0.08 per cent inulin respectively to the perfusion fluid resulted in the reappearance of these substances in the secreted fluid, but without concentration. Soluble starch failed to pass through the liver in amounts detectable by the iodine test. It therefore appears that the molecular size or aggregation limiting the entrance of lipoid-insoluble substances into the bile lies between those values for inulin and for soluble starch.

The liver's ability to concentrate the eriocyanin routinely administered to it in the perfusion fluid is in sharp contrast to its apparent inability to concentrate the several carbohydrates which were found to pass into the secretion. This latter fact suggests that the passage of these carbohydrates into the secretion is due to a filtration process.

The effect of light adaptation on dark adaptation. SELIG HECHT and CHARLES HAIG (by invitation). Laboratory of Biophysics, Columbia University.

We have measured for the human eye the influence of preadaptation to various intensities of white light on the course of the dark adaptation of a field 5° in diameter situated 30° nasally from the center, using a very low visual acuity as criterion.

With extreme violet light as stimulus, dark adaptation shows two stages. The first begins at once; it is rapid, and the violet color is always evident at the threshold. The second stage begins only after a definite time; it is slower than the first, and shows no color at the threshold.

As the intensity of light adaptation increases from 300 to 400,000 photons, the following changes are to be noted in dark adaptation. 1. The separation between the colored, first stage of adaptation and the colorless, second stage becomes clearer and more sharply defined. 2. The colored stage of adaptation increases in range, finally covering nearly 3 log units. Its speed, however, definitely decreases. 3. The range of the colorless adaptation also increases, but reaches a maximum of nearly 3 log units very soon and maintains both speed and range over most light adaptation intensities. 4. The time of appearance of the colorless, second stage of adaptation increases, the time being a sigmoid logarithmic function of the light adapting intensity.

Measurements with extreme red light show mostly the colored first stage of adaptation, and the behavior of the dark adaptation as a whole, following different intensities of light adaptation, shows much the same characteristics as the colored primary portions alone of the data with violet light.

We interpret the measurements to mean that the colored primary stage of dark adaptation represents mainly the function of the cones, while the colorless secondary stage of dark adaptation is a function of the rods.

The effect of oestrin and progestin on the reaction of the rabbit's uterus to pituitrin in vitro. GEORGE P. HECKEL (by invitation) and WILLARD M. ALLEN. Department of Obstetrics and Gynecology, The University of Rochester, School of Medicine and Dentistry.

Three types of reactivity of the rabbit's uterus to pituitrin in vitro have been observed: 1, contraction, obtained in normal oestrous animals and in recent castrates; 2, failure of response; and 3, relaxation. The conditions necessary for the latter two responses have not been fully explained.

The conditions necessary for these responses to pituitrin in vitro have been investigated by studying uteri from castrated rabbits which have been under the influence of oestrin, progestin, or combinations of the two from 16 to 21 days. Castrated, pseudopregnant, and unilaterally pregnant animals, untreated or treated with oestrin (progynon-B) were used as controls.

Uteri of animals castrated from 16 to 21 days were found to contract to pituitrin without having received any treatment. Castrates treated only with oestrin (progynon-B) reacted in a like manner, but to a more marked degree. Castrates receiving progestin alone failed to react (one case out of seven showed relaxation on the sixteenth day). Castrates receiving simultaneous treatment with oestrin and progestin all showed definite relaxation when pituitrin was added to the bath.

From these experiments it is concluded that when the rabbit uterus is under the influence of oestrin it contracts to pituitrin in vitro; when influenced by progestin it shows no response; but when it is under the influence of both oestrin and progestin it relaxes.

Cation permeability of erythrocytes in experimental adrenal insufficiency and in normal animals after plasma sodium depletion. A. H. HEGNAUER and E. J. ROBINSON (introduced by F. A. Hartman). Department of Physiology, Ohio State University.

Sodium, chloride and potassium concentrations of cat plasma and erythrocytes (in which Na is quantitatively the most important cation) together with the water contents have been studied in experimental adrenal insufficiency. The Na and Cl concentrations were decreased while the K concentration was increased in the cells. These changes were roughly proportional to those found in the plasma. It is concluded that the altered concentrations within the cells result from passage of cations across the erythrocyte membrane.

To determine whether this permeability phenomenon is peculiar to adrenal insufficiency, a further series of experiments was undertaken in which normal cats were given intraperitoneal injections of quantities of isotonic glucose with subsequent paracentesis. Although the plasma Na, Cl and K levels of these animals approximate those obtaining in relatively extreme adrenal insufficiency, the cell cation changes, while in the same direction, are much less marked.

Spontaneous variations in human gastric acidity. FRANCES A. HELLEBRANDT, RUBYE TEPPER (by invitation) and RUTH CATHERWOOD (by invitation). University of Wisconsin.

That the fasting stomach may continuously secrete a juice of moderately high acid value was suggested as a result of 55 observations on 6 normal

young adult women extending over periods of from 1 to 6 hours. Continuous intubation was therefore lengthened to include 12 consecutive day and night hours. Small fractional samples of the gastric contents were withdrawn every 30 minutes and tested for free and total acidity, using the microtechnique of Hollander. A total of 33 twelve hour observations were made, 12 during the daytime on 11 different subjects, and 21 during the night on 18 subjects. A single observation was protracted to 17 consecutive hours. In this instance aspiration was continuous, using the Wangensteen negative pressure method, and fluids were replaced by hypodermoclysis. The findings show that the normal, unstimulated stomach rarely demonstrates secretory quiescence. Spontaneous variations in acidity occur at irregular intervals both day and night. At the peak of these cycles, the acidity of the gastric contents of the fasting stomach may exceed that following the administration of a test meal. Contrary to the findings of Winkelstein, Chalfen, Henning and Norpoth, free acid may be abundantly present in the gastric juice secreted by normal young adults during sleep at night.

Studies on the blood flow of the kidney. J. F. Herrick, H. E. Essex, E. J. Baldes and F. C. Mann. Divisions of Experimental Medicine and Physics and Biophysics, Mayo Clinic, Rochester, Minnesota.

Data on the blood flow in the renal artery of the normal intact dog will be presented. These data will be compared with data obtained on the animals when anesthetized with ether and with amytal. A comparison of the blood flow in the renal vein with that measured concurrently in the renal artery will be reported. Results of observations on the following will also be given: 1, blood flow changes during digestion of food; 2, blood flow changes during exercise on the tread mill; 3, blood flow changes during the intravenous injection of: *a*, sodium sulphate; *b*, creatinine; *c*, urea; *d*, glucose; *e*, amyl nitrite. 4. Blood flow changes caused by stimulation, novocainization and sectioning of the renal nerves.

All blood flow measurements are made by the Thermo-stromuhr of Rein.

Influence of sympathetic nerves upon the effect of pituitary extracts on blood sugar in dogs. R. C. Herrin and H. F. Haney (by invitation). University of Wisconsin.

Various extracts of the pituitary gland, which had been prepared chiefly for their effects upon growth, luteinization or Graafian follicle formation, were injected intravenously in dosages representing from 1 to 5 grams of the dried gland. All extracts caused a 15 to 110 per cent increase in blood sugar. This increase would appear within 6 minutes after injection and persisted for 2 hours or more. Repeated injections usually gave no response by the third or fourth time, although the sugar tolerance remained normal. Following these experiments, in 5 dogs the splanchnic and vagal nerves were sectioned and both lumbar chains excised. These denervated animals, including 2 other such dogs, gave a different response to the injections of pituitary extracts. The elevation of blood sugar was delayed until 10 to 20 minutes after the injection and within 25 to 60 minutes the blood sugar had returned to normal or to a hypoglycemic level. All animals eventually showed a marked hypoglycemia. One animal died with all the signs of hypoglycemia shock about 3 hours after the injection.

One factor involved in the different response after the denervation is the inability of the animal to mobilize glucose from its glycogen. As shown by other workers, such animals show a lessened response to injections of epinephrine. Vagotomy did not change the response to the pituitary extracts.

In these denervated animals the sympathico-adrenal mechanism is handicapped in correcting hypoglycemia and the results therefore may be interpreted either as a demonstration of the pancreatropic principle in the pituitary which causes the secretion of insulin and the consequent lowering of blood sugar or as the same principle which normally causes elevation of blood sugar but which is now ineffective because of the denervation.

Studies on the mechanism of reflex acceleration of respiration through afferent fibers in the vagus nerve. CHARLES HILLENBRAND (by invitation) and T. E. BOYD. Loyola University.

Using dogs anesthetized with sodium barbital or nembutal, and with both vagi sectioned, we have confirmed the findings of Hammouda and Wilson (J. Physiol. **85:** 62, 1935) that continuous tetanization of the vagus below a cooled point may cause acceleration of respiration, instead of the stoppage or slowing seen when the nerve is left at room temperature; and that this reversal is not due to a simple reduction in frequency of impulses conducted.

While "augmentor" fibers apparently exist in the vagus, their section can hardly be responsible for the slow rhythm which follows double vagotomy. With one nerve sectioned, the other intact and gradually cooled to complete block, the respiration is progressively slowed, though the authors cited above consider that inhibitory fibers are selectively blocked at 8°. Without changing the frequency or strength of stimuli, a "reversal" of the reflex can be obtained with the nerve left at room temperature. This is done by a circuit so arranged that contact is made and broken by changes in the intrathoracic pressure of the animal during natural respiration. The vagus is tetanized, not continuously, but only when the lungs are inflated beyond a certain point. The amplitude of inspiration is reduced and the frequency increased. The acceleration is immediate. Similar effects can be produced by stimulation of the superior laryngeal nerve in the same manner.

These observations indicate that respiration can be accelerated by a periodic inhibition which cuts short inspiration, as the normal (inhibitory) inflation reflex does (Adrian, J. Physiol. **79:** 332, 1933). The slow breathing of vagotomized animals may therefore be attributed to the loss of this reflex.

A mechanism of dehydration in diabetes. H. E. HIMWICH and J. F. FAZEKAS (by invitation). Departments of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

A well-known mechanism of dehydration in diabetes finds its genesis in ketonuria. The ketone substances are eliminated as sodium salts, and due to the loss of sodium, water also must leave the body. In the present experiments dehydration without ketonuria was studied. Partly depancreatized dogs were given carbohydrate, fat and protein in amounts sufficient to maintain caloric equilibrium, and insulin in doses adequate to prevent glycosuria. Serum protein, total base of the serum, oxygen

capacity as well as nitrogen, glucose, total base and ketone substances of the urine were determined. Then the glucose content of the diet was increased producing a glycosuria uncomplicated by ketonuria. With the glycosuria, the urinary volume increased as did that of the total base of the urine. The animal sustained a loss of weight due to dehydration as could be told by the increased serum protein and raised oxygen capacity. Subsequently, by increasing the salt of the diet, the body was rehydrated as the salt deficit was made up. Here, then, is a mechanism of dehydration not dependent on ketonuria but on a glycosuria.

The anterior border of the monkey's (Macaca mulatta) motor cortex and the production of spasticity. MARION HINES. Department of Anatomy, Johns Hopkins Medical School.

A strip of cortical tissue about 3 mm. in width, passing through the center of the superior precentral sulcus, duplicating the curvature of the central fissure, ending on the lateral surface in the vicinity of the inferior precentral fissure, and extending on the medial surface to the sulcus callosus marginalis, was removed unilaterally and bilaterally in 7 monkeys. Examination of serial sections, fixed and stained, proved the cortex ablated was taken from area 4 of Brodmann.

Study of the surviving animals showed unmistakably, contralateral to the lesion, not only the signs of partial motor impairment, which rapidly regressed, but also brisk, spreading tendon reflexes, increased resistance to passive movement (particularly in the extensors), and clonus,—all classical signs of spastivity. And these persisted. Contractures also developed if this lesion were extended posteriorly to include a larger portion of the motor field. These spastic animals never showed forced grasping.

On the other hand, bilateral removal of the area originally delimited by Richter and Hines (1932) for the production of forced grasping (Brodmann's area 6) did not produce either motor impairment nor any evidence of spasticity. However, the subsequent extension of this lesion to include the anterior border of area 4 added to the condition of forced grasping, the signs of spasticity enumerated.

Clearly, therefore, forced grasping and spasticity result from interference with the activity of the cerebral cortex in two regions, anatomically discrete and cyto-architecturally distinct. The nervous tissue whose ablation produces forced grasping is concentrated in Brodmann's area 6; and that, whose removal results in spastic phenomena is most strongly represented in the anterior part of Brodmann's area 4, the area giganto-pyramidalis of Economo and Koskinas.

Consequently, forced grasping and spasticity are not integral parts of one syndrome of the premotor area. Rather, these two conditions represent a loss of important inhibitory functions having independent origin within the larger motor area of the cortex cerebri.

The effect of splanchnic resection on the metabolic response to insulin in a case of severe diabetes. FRED A. HITCHCOCK and GEORGE M. CURTIS. Departments of Physiology and Medical and Surgical Research, The Ohio State University.

A study has been made of the basal metabolism and of the metabolic response to insulin in a patient with severe diabetes, before and after bilateral splanchnic resection. All determinations of the metabolism were

carried out by means of the Tissot-Haldane technique. On each experimental day, two or three basal periods were first run. Fifteen or twenty units of insulin were then administered subcutaneously and the respiratory metabolism was then followed continuously for approximately one and a half hours. Three such experiments and two control determinations, in which normal saline instead of insulin was injected, were carried out before the operation. Total urinary nitrogen was determined before and after the injection in each experiment. Bilateral splanchnic resection was performed by the supra-diaphragmatic approach through the posterior mediastinum. The operation was in two stages; the left nerve was first sectioned and three months later the right nerve was sectioned. In the interval between the two parts of the operation one experiment was performed. A little more than three months after the second operation a series of three experiments were run. The results show that there was a marked change in the basal oxygen consumption following the operation. The average preceding the operation was 220.4 cc. per minute. In the period between the two parts of the operation, the basal O_2 consumption dropped to 197.0 cc. per minute and the average three months after the second half of the operation was 240.6 cc.

The insulin requirement following the operation was markedly decreased. Preceding the operation the injection of one unit of insulin caused the oxidation of 197 mgm. of carbohydrate during the first hour and a half. After the operation one unit of insulin caused the oxidation of 258 mgm. of carbohydrate in a similar length of time. The sparing effect on protein metabolism of the increased oxidation of carbohydrate resulting from insulin administration was increased after the operation.

On the mechanism of the "Berger rhythm" in normal man and in general paretics. HUDSON HOAGLAND. Biological Laboratories, Clark University, and the Worcester State Hospital.

"Spontaneous" electrical oscillations in the central nervous system may be relaxation oscillations, with frequencies directly proportional to the speed of underlying chemical events (cellular respiration, for example).

The "ten-a-second" alpha rhythm from the human occipital cortex should thus obey the Arrhenius equation as a function of internal body temperature.

$$\text{Frequency} = kv = e^{-\mu/RT} + c$$

where v is the velocity of the underlying chemical system, e the base of natural logarithms, R the gas constant, T the absolute temperature, k and c constants, and μ the critical thermal increment.

Ten human subjects were given an average of four diathermy treatments each, temperatures being elevated usually to 105°F. in from one to two hours. At fifteen minute intervals, with heating current off, records of the alpha rhythm were determined by means of an amplifier and ink-writing undulator. The alpha frequencies were averaged for each temperature. The Arrhenius equation was found to describe the data satisfactorily. Of the ten subjects, three were normal controls, one was a mentally normal multiple sclerosis out-patient, and six were general paretics. The normals, the multiple sclerosis patient, and the two least affected general paretics gave values of μ of 8,000 $\pm$ calories. Two other

general paretics gave 11,000 ± calories and two gave 16,000 ±. This last value corresponds to an increase of from approximately 10 to 13 cycles per second for a rise of roughly 6°F. (3°C.). These three values of critical increments have been found repeatedly by various workers to be associated with respiratory processes in cells where direct measurements of O_2 consumption and CO_2 production have been made. In some respiratory investigations it has been found possible experimentally to shift the μ from one of these values to another of the three. It has elsewhere been suggested that these critical increments may correspond to links in the respiratory chain such that under certain conditions one or the other may predominate as a pacemaker by acting as the slow master reaction in the respiratory chemical sequence. The pathological condition in the paretics, therefore, appears to shift the pacemaker involved in respiration of the cells of the occipital cortex.

On the nature of intestinal absorption of amino acids. RUDOLF HÖBER (introduced by H. C. Bazett). Department of Physiology, University of Pennsylvania.

Experiments on rats have brought to evidence that the intestinal absorption of amino acids, as compared with other organic substances, goes on with higher speed than could be expected from the diffusion rates. It is concluded that a special physiological factor is involved.

Delayed death in relation to barbiturate structure. HARALD G. O. HOLCK and PAUL R. CANNON. Departments of Physiology and Pathology, University of Chicago.

Following the administration of suitable doses of isopropyl-beta-brom-allyl barbituric acid (nostal) to rats, the animals apparently recovered from acute depression within a few hours; but most of them died later, usually on the second or third day, from pulmonary edema, generally accompanied by pneumonia and fatty degenerative changes in the liver, kidneys, heart and lungs. Such delayed death occurred occasionally even from subhypnotic doses of nostal. This delayed death also has been attributed to pulmonary complications by Fitch and Tatum (J. Pharmacol. Exper. Therap. **44**: 325, 1932). The delayed deaths were not due to any unusual sensitivity to bromides, nor to the acidity of the hydrobromic acid liberated, because sodium allurate (isopropyl-allyl barbiturate) with sodium bromide or with hydrobromic acid did not cause delayed death.

Adding one CH_2 group to the isopropyl group of nostal (giving pernoston), or methylating one nitrogen in nostal (eunarcon), gives compounds which only exceptionally caused delayed death.

The chlorine homologue of nostal and dichlor-allyl barbiturate also caused delayed death, although less readily; but no such death was seen after the administration of the chlorine homologue of pernoston.

Delayed death did not follow the administration of the closely related isopropyl-allyl (alurate), diallyl (in dial), or isopropyl-ethyl (ipral) barbiturates; nor could it be demonstrated after administering one of the supposed early decomposition products of nostal, namely, isopropyl-acetonyl barbiturate. Nine other barbiturates also were ineffective in causing delayed death in the rat (amytal, barbital, evipal, neonal, ortal, pentobarbital, phanodorn, phenobarbital, and sandoptal).

Periodic fluctuations in motor response to uniform electrical stimulation of the cerebral cortex in monkeys. CARL IVER HOVLAND (by invitation) and J. G. DUSSER DE BARENNE. Laboratory of Neurophysiology, Yale University School of Medicine.

In their study concerning the effect of hyperventilation on the excitability of the "motor" cortex of cats under "Dial" anesthesia Brody and Dusser de Barenne (1932) reported that upon faradic stimulation under constant experimental conditions there appear "waves" of cortical excitability, reflected in the magnitude of the responses. With stimulations given at intervals of one minute, the period of these fluctuations was from two to five minutes. After pointing out the similarity of these waves to the well-known, more rapid fluctuations found in mental activities, such as attention, the authors suggested the possibility that the deep anesthesia "might result in a lengthening of normally much shorter fluctuations in cortical activity."

Thus it became of interest to repeat these experiments on monkeys (macacus), both narcotized and non-narcotized. A focus of the precentral "motor" arm area was electrically stimulated by impulses from a thyratron circuit; the stimulations, uniform with respect to duration, intensity, pulse frequency and pattern frequency, were applied at one minute intervals, rigidly controlled by a mechanical device. This interval avoids interference of "facilitation" and "extinction." The motor responses were recorded isotonically on smoked paper. In both the anesthetized and the unanesthetized animals periodic fluctuations in amplitude and latency of response were present. The most characteristic period of these "waves" was four minutes; in the non-narcotized animals the periodicity was less regular, presumably due to extraneous factors. To be non-committal as to the level at which these "waves" originate, we prefer, at present, to speak of periodic fluctuations in motor response, rather than in cortical excitability.

Search of the literature has disclosed evidence in previous researches of a four minute periodicity unmentioned by the investigators. Protocols of Pavlov show such variations in conditioned salivary responses. In the work of Galli and Gemelli on reaction times of human subjects and in that of Wiersma on sensory thresholds similar cycles are present. Experiments (unpublished) on galvanic skin reactions of human subjects to weak electric shocks have likewise revealed "waves" of four minutes' duration (Hovland). (Lantern slides.)

The nature of the abnormalities in measurements of the colligative properties of egg yolk. EVELYN HOWARD. Department of Physiology, Johns Hopkins School of Medicine.

The question as to whether the yolk of the hen's egg is in osmotic equilibrium with the blood of the hen or with egg white has been answered by two contradictory sets of evidence. One group of investigators reports freezing point determinations and vapor pressure measurements which indicate that egg yolk has a freezing point corresponding to approximately 0.98 per cent NaCl, while certain cryoscopic and vapor pressure techniques used by the author and others indicate that the value should be 0.73. The latter value is equivalent to the effective molar concentration of egg white.

Present determinations consist of studies of the boiling point of egg yolk dialysates. Measurements of the boiling point of dialysates during high speed stirring, with suitable precautions to avoid the presence of interfering volatile solutes, gave an average value of 0.730 per cent NaCl as equivalent to the concentration of egg yolk. Boiling point measurements with the Cottrell technique in which the interphase is allowed to break spontaneously gave a higher value. Analysis of the various types of technique hitherto used indicates that in egg yolk or its dialysate, measurements of the solvent's escaping-tendency involving rate of molecular transfer across a mechanically static interphase, or one which is being renewed at a rate below a certain critical level, uniformly give a high value, whereas measurements across a rapidly breaking and reforming interphase give the lower value. This property is presumably due to the presence of an unusually surface-active substance, provisionally named *cryocryptin*. The possibilities of the action of this substance in limiting or conditioning the osmotic transfer of water across cell membranes remain to be studied, but may prove to be of general importance in certain types of cellular regulation of water transfer.

Spike action potentials from fiber tracts in the spinal cord of the cat. HOWARD A. HOWE and DEAN CLARK (introduced by H. S. Gasser). Department of Physiology, Cornell Medical College, New York City.

Single break shocks from a Harvard inductorium were applied to the pyramids in the cat's brain. Direct stimulating contact with the ventral surface of the pyramidal tracts was obtained by means of bipolar silver electrodes imbedded in a small bakelite block which was fitted rigidly into a trephine hole through the basal portion of the occipital bone. Leads were obtained through coaxial electrodes made with hypodermic needles and introduced into the substance of the spinal cord through drill holes at various levels in the cervical vertebrae. The resulting action potentials were visualized by means of the cathode ray oscillograph. These experimental procedures involved minimal disturbance to the circulation and temperature of the cord.

In form and conduction velocity the potentials thus recorded were very similar to the spikes commonly observed in peripheral nerves. Further likeness was seen in a resistance to narcosis and asphyxia, greater in degree than holds for central nervous function in general. Rapid exsanguination of the animal through the left ventricle of the heart led to disappearance of the potentials after 5 to 10 minutes, in contrast to another group of potentials which disappeared within two minutes and were taken to represent post synaptic activity in the cord.

Conduction velocities were measured between two sets of leads distant about 20 and 50 mm. respectively from the point of stimulus. Possible errors due to spread of stimulus were thus avoided. Calculated conduction velocities fell into two groups, at 64 to 80 meters per second, and 104–117 meters per second. The former were seen after shocks of medium strength. In one experiment a spike showing high amplitude and little temporal dispersion was correlated with the presence of the lead in the lateral column of the cord, and was thought to represent a pyramidal tract potential. The second group of more rapid potentials was obtained with stronger shocks. These were interpreted as the activity of larger fibers separated from direct contact with the stimulating electrodes by considerable tissue.

The blood sugar level following physostigmine and atropine. M. CAROLINE HRUBETZ (introduced by E. H. Scott). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

A total of 450 observations were made on normal-fed rats. Approximately 50 observations were made for each point on the curve. After the subcutaneous administration of physostigmine in the dosage of 0.25 mgm. per kilo, there was a sharp rise in the blood sugar level, the greatest change occurring in one hour. In two hours the curve had returned to the normal level.

The same dose of physostigmine was injected into another series of rats 30 minutes after atropine had been administered in the dosage of 20 mgms. per kilo. The blood sugar level, followed for 1½ hours, showed no change. Atropine, therefore, does prevent the rise in the blood sugar level after physostigmine.

Absinthe and the blood sugar level. M. CAROLINE HRUBETZ (by invitation) and F. H. PIKE. Department of Physiology, Columbia University. (Read by title.)

Twenty-nine experiments were done on rabbits. A five per cent alcoholic solution of the oil of absinthe (identical with that used in earlier experiments on cats) was administered by vein in doses ranging from 0.13 cc. to 0.6 cc. (0.03 to 0.06 cc. per kilo of body weight), some of which were of convulsive magnitude, and others of sub-convulsive magnitude. Bleedings were begun immediately after the convulsion, or after the smaller signs which appeared when the dose was sub-convulsive, and continued at intervals for an hour or more. In 19 cases, there was a rise in the concentration of sugar in the blood, in three cases a fall, and in seven, no change. Usually the sugar level had returned to normal within an hour. (Normal means preconvulsive level for each individual animal.) No correlation is apparent between the size of the dose and the subsequent change in the concentration of sugar.

All animals showed signs of marked excitation of the autonomic nervous system. These signs were of either craniosacral or thoracico-lumbar origin, or sometimes from both at once, or in alternation. In all cases, the autonomic response preceded the somatic response, as shown in the movements of the skeletal muscles.

Any protective function of a convulsion does not seem a necessary conclusion from these data.

Treatment of undescended testes (cryptorchidism) with an extract of human placenta. O. LEONARD HUDDLESTON (introduced by Maurice H. Rees). Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, Colorado.

A limited clinical study has been made on a series of fifteen boys, all preadolescent except one, in which a maldescent of one or both testes was present. The chief aim of the investigation was to determine the efficacy of placental prolan (APLH) as a therapeutic agent in the treatment of cryptorchidism. Treatment consisted of subcutaneous injections of an alcohol-insoluble extract of human placentas prepared by the Collip method of extraction. The injections were given daily for a period of 10 or 15

days and then reduced to triweekly injections. The customary dose employed was 1 cc. containing a prolan equivalent of either 25 or 50 grams of fresh placenta. Bioassay showed the extract to possess a potency that ranged from 50 to 100 rat units per cc. The initial response induced by the injections was found to be comprised of one or more of the following alterations: 1, increase in the mobility of the testes accompanied by a thickening and lengthening of the spermatic cord; 2, migration of the testes toward the scrotum; 3, testicular enlargement; 4, elongation of the scrotum, frequently combined with an intense pink or reddish coloration of the scrotal skin. With continued treatment, other changes such as an enlargement of the penis, growth of pubic hair, turgescence and rugosity of an enlarged scrotum, genital hypersensibility leading to rather frequent penile erection, were subsequently observed in some cases. Analysis of the data shows the following results: 1, considerable variation in the rapidity and extent of genital response was exhibited by different patients; 2, spontaneous descent of the testes occurred in two cases; 3, complete correction of the abnormal position of the testes was secured in 40 per cent of the cases; 4, a partial correction, consisting of an enlargement or descent of the testes was obtained in 40 per cent; and 5, complete failure resulted in 20 per cent of the cases. Five of the patients are still receiving treatments so that the percentages may be altered eventually.

Demonstration of the polyelectrophysiograph. O. Leonard Huddleston (by invitation), Richard W. Whitehead and B. E. Moritz, Jr. (by invitation). Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, Colorado.

The polyelectrophysiograph is a compact portable instrument which has been designed primarily for teaching purposes and research work in physiology and associated subjects at the medical school. It is believed however, that the principles employed in its construction may be utilized to build a small simplified instrument which would be suitable for general clinical usage as well as for class room demonstrations. Our machine has been constructed in such a way that, when used with suitable electrodes, it may be employed to detect, measure or record any small electrical current which may develop in any organ or tissue such as the heart, nerve, muscle or gland tissue during functional activity. The electrical currents may be observed as either standing or transient waves or may be photographed. In addition to the visual study of any pulsating electrical current the machine may be used to convert the oscillations into auditory waves so that they may be heard either combined with the optical examination or entirely separate. In general, the instrument is composed of two cathode ray tubes which are controlled by their respective amplifiers and sweep circuits. This portion of the machine is operated by the ordinary 110 volt 60 cycle current and may be used as a regular cathode ray oscillograph. A high gain direct current amplifier may be combined with the oscillograph unit when it is desirable to obtain greater amplification. The instrument contains an adjustable standardization apparatus which may be used either to measure the voltage of a bioelectric current or to adjust the sensitivity of the machine. If one wishes to listen to the electrical vibrations associated with some physical or physiological vibratory activity it is only necessary to switch them into the power amplifier which operates the loud

speaker. The machine will be employed to demonstrate such physiological phenomena as the electrocardiogram, electrophonogram, electroencephalogram and possibly a few principles pertaining to acoustics if time permits.

Effect of elevation of temperature within the physiological range on outlying bone marrow. CHARLES HUGGINS, W. J. NOONAN (by invitation) and B. H. BLOCKSOM, JR. (by invitation). Department of Surgery, University of Chicago.

Soon after birth in mammals and birds hemopoietic bone marrow retreats from the tips of the limbs and tail and is replaced by fatty marrow. The cause of this regression has been analyzed and is due, at least in part, to a thermal decrement in the extremity marrow chamber.

Albino rats were used throughout and all operations carried out under ether anesthesia. In one series, the distal portion of the tail was denuded of skin and sutured in the peritoneal cavity as a chronic preparation; a return of red marrow in older rats and a preservation in younger rats was found in the area where temperature was elevated. In a second series the tail was amputated in new born rats and transplanted to the foot and abdomen of the mother; after 60 days the transplants in the foot contained only yellow marrow, while the abdominal transplants contained red marrow. In a third series, the animals were kept for 28 days in an incubator at 35°C. The marrow changes here also will be reported.

Cord potentials in acute and chronic spinal cats. JOSEPH HUGHES (by invitation), GRAYSON P. MCCOUCH and WINIFRED B. STEWART (by invitation). Pennsylvania Hospital for Mental and Nervous Diseases and Department of Physiology, University of Pennsylvania.

Last year evidence was presented of a correlation in acutely spinal cats between inhibition of the ipsilateral flexion reflex by a preceding contralateral volley and reduction of the slow negative components of the cord potential induced by the ipsilateral volley. The present study extends the observations to chronic preparations and to the fate of the slow positive component of the cord potential.

In the acutely spinal cat, if the ipsilateral shock falls during the negative component of the preceding contralateral volley, the reflex is frequently facilitated and associated with preservation and occasionally augmentation of the negative components. In the chronic spinal cat such early second shocks usually result in reflex inhibition, reduction of early, and abolition of late elements of the negative complex. In the chronic cat reflex inhibition and the positive component of the cord potential are deeper and longer and the negative components briefer than in the acute preparation. In both acute and chronic animals an ipsilateral volley during the positive component from a preceding contralateral volley permits less positivity to develop than obtains in either response alone. This result may be related to the effect observed by Eccles and Sherrington (Proc. Roy. Soc. **109:** 91, 1931) of an interpolated ipsilateral volley in reducing the inhibition of a subsequent ipsilateral by a preceding contralateral volley. If the ipsilateral volley fall during the negative complex following the contralateral volley, it induces reduction of the subsequent positivity in the acute cat, augmentation of it in the chronic.

These results accord well with the view recently advanced by Eccles in

the case of the superior cervical ganglion that the slow potentials are generated in perikaria. They do not exclude an alternative explanation based upon axone potentials involving a selective distribution of large and small internuncial fibers.

The influence of thyroid feeding and thyro-parathyroidectomy on bone regeneration. I. F. HUMMON, JR. (by invitation), MARY C. PATRAS (by invitation) and R. D. TEMPLETON. Departments of Physiology, Medicine and Radiology, Loyola University School of Medicine, Chicago.

This study was conducted upon albino rats, 33 receiving a standard diet, and 41 a vitamin B deficient diet. These animals, 149 to 177 days old, were taken from the Loyola colony, which received Fox chow ad libidum and beef hearts, bread, and cabbage weekly. Littermates, the same age and sex, were used for comparison whenever possible.

The standard diet consisted of casein 18 per cent, starch 47 per cent, wet yeast 15 per cent, unsalted butter 15 per cent, inorganic salt mixture (Harris) 3 per cent, and cod liver oil 2 per cent. The vitamin B deficient diet was made by substituting starch for 10 per cent of the yeast in the standard diet. For thyroid feeding 0.05 per cent desiccated thyroid was added to the respective diets.

On the 18th day of the experiment 11 of the standard diet group and 14 of the vitamin B deficient group were thyro-parathyroidectomized. Also 11 of the standard group were started on thyroid feeding and 11 continued as controls. On the 27th day holes were drilled in the lateral cortex of the right femurs of all animals and 14 of the vitamin B deficient group were started on thyroid feeding, the remainder continuing as deficiency controls. The thyro-parathyroidectomized, control, and thyroid fed animals of the vitamin B deficient group survived 35 to 66, 31 to 52, and 30 to 47 days respectively after bone drilling. The standard diet group was sacrificed 85 days after bone drilling. Periodic radiographs were made to follow bone regeneration and a final radiograph was made of the excised femurs.

The results show that thyroid feeding accelerated while thyro-parathyroidectomy retarded bone regeneration in the standard group. In the vitamin B deficient group the thyroid fed animals showed very poor bone regeneration during their short survival period; the controls, living longer, showed better regeneration; and the thyro-parathyroidectomized animals, surviving the longest, showed the best regeneration.

The nature of the influence of temperature on the osmotic properties of the erythrocyte. M. H. JACOBS, H. N. GLASSMAN (by invitation) and A. K. PARPART (by invitation). Departments of Physiology, University of Pennsylvania, and Princeton University.

The greater resistance of the erythrocyte to osmotic hemolysis at high than at low temperatures has been variously attributed to an increased hydroxyl ion concentration of the external medium (Jarisch), to a more rapid escape of osmotically active substances from the cell (Ponder), to a reduced susceptibility of the cell to mechanical injury (Lepeschkin), and to a reduction of the internal osmotic pressure through a change in the relative base-binding powers of hemoglobin and carbonic acid (Jacobs and Parpart). According to the last theory temperature changes under otherwise constant external conditions should be associated with reversible

volume changes of the cells. Such changes can readily be demonstrated by the optical method of one of the authors (Parpart), the cells (ox) shrinking on raising the temperature and approximately resuming their original volumes on again lowering it. According to this theory also, but not according to the leakage theory, cells brought to approximate osmotic equilibrium in a properly chosen hypotonic solution at 40°C. should undergo further swelling and hemolysis on lowering the temperature. This effect can readily be demonstrated in NaCl solutions with erythrocytes of the dog and the ox. The failure of Lepeschkin to observe it with human erythrocytes is doubtless to be explained by the relatively rapid changes which these cells, in common with those of the rat, the guinea pig and probably other species, undergo in hypotonic solutions of NaCl. These changes may plausibly be attributed to a progressive loss of potassium from the cells. Such a loss, though not primarily responsible for the increased resistance at 40°C., would nevertheless tend to preserve the resistance established by other means. In support of this view is the fact that in buffered solutions of KCl the erythrocytes of man, the rat and the guinea pig behave in essentially the same manner as do those of the dog and the ox in similar solutions of NaCl.

Restitution of function after cortical injury in monkeys. C. F. Jacobsen, F. V. Taylor and G. M. Haslerud (introduced by J. F. Fulton). Laboratories of Primate Biology, Yale University, New Haven.

Theories of restitution after cortical injury generally postulate one of the following types of adjustment: 1, the subject learns to adjust without the lost function; 2, some other part of the nervous system not previously concerned with the activity vicariously assumes that function; and 3, there is a dynamic reorganization within a *partially* destroyed system. The experiments to be reported are concerned with the last category. Bilateral removal of motor and premotor areas of adult monkeys is followed by enduring paralysis: voluntary movement is lost and postural adjustments are similar to those of thalamic preparations (Fulton and others). However, the same lesion in infant monkeys causes a paralysis of short duration, and the animal soon runs, climbs, and manipulates objects while the adult is completely helpless. There remains, however, in the infant some deficit of finely adapted movement of digits and postural abnormalities (Kennard).

Extirpation of frontal association areas from adult animals causes total impairment of "recall" (delayed response technique), although recognition memory is little disturbed. But in contrast to the considerable motor recovery in infant monkeys, there is no recovery after lesions of the association areas. Impairment of "recall" is as severe as in adult subjects. It seems probable that this difference in recovery after motor and frontal area lesions arises from partial destruction of a dynamic system in the former instance, and complete removal in the latter. Thus the cortical motor area is only one component of the postural-locomotor system, and, under certain conditions, the remaining parts can carry on. Recovery is more complete when injury occurs before this cortical component has been functionally integrated with subcortical mechanisms. On the other hand, no other part of the nervous system can mediate the functions of the frontal association areas. If this region is removed completely, no recovery follows, and it is of little consequence whether such injury occur in infancy or adult life. Further evidence on the effects of disturbing a

functional system, obtained with subtotal injuries of the frontal association areas will be presented.

The influence of skeletal muscle tension and relaxation on blood pressure. EDMUND JACOBSON. Physiological Laboratory of the University of Chicago.

Simultaneous registration of muscular states and of blood pressure is effected during periods of about one and one half hours in human subjects lying upon a couch. By methods described previously, using two amplifier string-galvanometer systems, records of action-potentials are secured simultaneously from two muscle regions, most often the flexors of the right arm and of certain muscles in the abdominal wall. An alternative is to record from four different muscular regions by taking samples from each region at regular brief intervals. Blood pressure is taken from time to time with a Tycos self-recording sphygmomanometer. The results can be graphed conveniently, plotting peak microvoltage per second from each region as well as systolic and diastolic blood pressures against time.

The investigations to be reported have been carried out on twenty-two subjects, of whom fifteen showed normal pressure while seven suffered from chronic vascular hypertension.

It is well known that during the first minutes after lying down the blood pressure tends to fall. In records begun about fifteen minutes subsequent to lying down, a further fall both in systolic and diastolic pressure commonly occurs provided that skeletal muscle contraction is in the neighborhood of zero in the skeletal muscular regions tested. If there is striking failure to relax in various muscle-groups, the blood pressure commonly does not fall markedly during the stated interval, but may even rise.

Tonus in the mammalian ventricle. VICTOR JOHNSON and LOUIS N. KATZ. Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Simultaneous optical records of 1, left intraventricular pressure; 2, right intraventricular pressure, and 3, ventricular volume, were made upon barbitalized dogs with the chest open, using artificial respiration. Wiggers manometers and a glass oncometer, equipped with segment capsules, were used. One manometer cannula was inserted through the left subclavian artery, through the aortic orifice, into the left ventricle. Another was inserted through a slit in the right auricle, through the auriculoventricular orifice, into the right ventricle.

The heart rate was kept constant at all times by slowing the spontaneous rate with light vagus tetanization, or slow injection of a dilute mecholin solution, and driving the heart with a Lewis interruptor, by means of electrodes secured to the right auricle.

Comparisons of the pressures in both ventricles, and the ventricular volume at the end of diastasis were made under various experimental conditions. In a few instances, upon occlusion of the lower thoracic aorta, with a consequent elevation of intra-aortic pressure and improved coronary circulation, the ventricular volume diminished despite an elevation of the distending pressure in both ventricles. This is interpreted as an increase in ventricular tonus. In some cases, toward the close of an experiment, and shortly before the heart failed, the initial left and right intraventricular

pressures fell, while the ventricular volume increased. This is interpreted as a decrease of ventricular tonus.

In most instances of occlusion or release of the aorta, and in other experimental procedures, such as the injection of saline solution or adrenalin, left and right intraventricular pressures and ventricular volume changed in the same direction. Such changes cannot be interpreted as tone changes.

The saponifiable and non-saponifiable matter in bile as affected by diet. K. K. JONES and E. A. SEARING (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

This experiment was carried out to ascertain the effect food has on the relation between the saponifiable and non-saponifiable part of bile fat.

Total hepatic bile was collected from bile fistula dogs operated according to the method of Rous and McMaster. This bile was analyzed by converting all the fat into soaps by boiling an alkaline solution of bile with potassium hydroxide. From this solution the non-saponifiable lipids were extracted with petroleum ether, and from the acidified residue the fatty acids were separated by similar extraction.

The average of 17 analyses of bile when no food was given the dogs gave a non-saponifiable figure of 50 mgm. per 100 cc.; a saponifiable figure of 488 mgm. per 100 cc. and a ratio non-saponifiable to saponifiable of 1:9.8.

When a diet rich in carbohydrate is fed, 14 analyses average 21 mgm. per 100 cc. non-saponifiable, and 344 mgm. saponifiable, a ration of 1:16.4. Meat fed alone similarly gives (in 11 instances) figures of 24 and 328 mgm. respectively with a ratio of 1:13.5. Meat, plus the addition of 40 to 50 per cent vegetable oil in 9 analyses, gives a slight rise in all values 27 mgm. of non-saponifiable, 426 mgm. saponifiable and a ratio of 1:15.8. Liver and bread diet decreases (in 7 instances) the saponifiable matter with figures of 27 mgm. non-saponifiable, 328 saponifiable and a ration of 1:12.4.

Compared to this human fistula bile shows 93 mgm. non-saponifiable, 196 saponifiable and a ration of only 1:2.1.

Observations on the analysis of the saponifiable and non-saponifiable matter in bile. K. K. JONES and R. O. SHERBERG (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago. (Read by title.)

The study of the lipid chemistry of bile during the past five years has yielded the following facts:

1. A complete saponification of the fats in bile is obtained by boiling under a reflex condenser a solution of bile in 50 per cent alcohol containing 10 per cent of potassium hydroxide.

2. The non-saponifiable matter can be readily extracted from this alkaline solution by three changes of petroleum ether.

3. The extraction of fatty acids by means of petroleum ether from this solution after acidulation with phosphoric acid separates the bile fatty acids from bile salts satisfactorily, but fails to remove the hydroxy fatty acids present.

4. The fatty acids from freshly secreted bile contain 10 per cent or more volatile fatty acids calculated as butyric acid, this being the chief volatile acid.

5. The fatty acids from fresh bile contain 50 per cent or more of highly unsaturated acid. According to the iodine number and the formation of the octa bromide this fatty acid is almost wholly arachidonic acid which Brown (J. Biol. Chem. **80:** 455, 1928) finds is the predominant unsaturated fatty acid in hepatic lipids.

6. Arachidonic acid oxidizes readily on standing and especially fast in alkaline solution. The oxidized fatty acid separates from petroleum ether extracts of bile fatty acids as a fine white powder which on exposure to the air forms a transparent varnish. In pure form it gives the theoretical molecular weight of 440.

7. The presence of this oxidized fatty acid interferes seriously with the quantitative extraction of the fatty acids from bile. It may be performed in the bile. A small amount dissolved in bile fatty acid causes the acids to "creep" making it difficult to handle.

Chronology of certain physical changes of puberty. FREDERIC T. JUNG and ARTHUR L. SHAFTON (by invitation). Department of Physiology and Pharmacology, Northwestern University, and the Illinois Soldiers' and Sailors' Children's School.

In examining 169 normal boys, special attention was given to a mass of mammary tissue, the subareolar node, which appears under each nipple early in puberty, rapidly grows to a maximum size, and then regresses slowly through a period of several years. This probable history of individual nodes is deduced from the facts that 1, the greatest percentage of ++++ nodes, and the greatest average height of the nipple above the surrounding skin, were found in the 14.25 age-group, and that 2, in older age-groups the nodes were found in 100 per cent of cases but were much reduced in size.

As a rule the change in voice to a lower pitch occurred before the subareolar node reached its maximum size. It was also usual for the pubic hair to appear before the mammary enlargement began. Finally, it was more common to find a voice classified as low in the complete absence of pubic hair than to find a high, prepubescent voice with the pubic hair advanced beyond the ++ stage. Hence the usual sequence of events is 1, change of voice; 2, growth of pubic hair, and 3, attainment of maximal size by the subareolar node. The fact that this order is subject to nearly every possible combination of changes, and that one may find marked disparity in size between right and left nodes, must be taken into account in attempts to explain this phenomenon.

Further observations on the coronary circulation. LOUIS N. KATZ, KENNETH JOCHIM (by invitation) and ANNE BOHNING (by invitation). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, Illinois.

The work on coronary circulation previously reported by us has been criticized on the ground that the septal branch of the left descending coronary artery was not perfused in our experiments. While we had made certain in previous experiments not to omit the perfusion of any major branch of the coronary vessels, we, nevertheless, felt it desirable to repeat these experiments substituting Anrep's technique for cannulating the coronary arteries for the technique which we had previously employed. The present study confirms our previous results.

The chief conclusions which this study indicates are:

1. The coronary sinus outflow varies in the opposite direction to the coronary inflow when the vigor of the heart beat is altered at constant heart rate.

2. The thebesian channels can serve as a portal of entry as well as a portal of exit for the coronary vascular system.

3. The partition of coronary outflow between the coronary sinus and the thebesian channels of the right and left hearts is quite variable. Very little of the blood of the right circumflex coronary artery drains into the coronary sinus.

These results show that observations made on changes in the coronary sinus outflow in the beating heart do not necessarily indicate the reaction of the coronary vessels. The analysis of the changes in coronary caliber requires the use of coronary inflow measurements (or total coronary outflow) and the elimination of the extravascular effect of the beating heart. This latter we have accomplished by throwing the ventricles into permanent fibrillation while perfusing the coronary arteries at a constant high pressure level (around 120 mm. Hg).

Modification of reflex contractions of the tibialis anticus by administration of sodium sulphide and cyanide. WILLIAM KAUFMAN (introduced by Robert Gesell). Department of Physiology, University of Michigan.

The tibialis anticus muscle was reflexly activated by induction shocks of constant strength and rate and the reaction to intravenous injections of sodium sulphide after denervation of the carotid sinuses was studied.

The effects of intravenous injections of sodium sulphide on the reflex contraction can be divided into four stages, depending on the amount injected. Stage I. Slight increase in the reflex amplitude followed by a return to the pre-injection level. Stage II. Depression of the reflex amplitude preceded by an initial increase in reflex amplitude and followed by a period during which the reflex increases above the depression level to a value less than, equal to, or greater than the pre-injection level. Stage III. Total suppression of the reflex preceded by an initial augmentation of the reflex amplitude followed by the reappearance of the reflex contraction which is of gradually increasing amplitude. Stage IV. Initial increase of the reflex amplitude followed by a permanent suppression of reflexes.

A striking parallelism existed between the action of the reflex contraction amplitude of the tibialis anticus muscle and the changes in respiration due to central injections of sulphide reported by Winder and Winder (1933).

Localized chemical stimulation of the carotid gland by intra-arterial injections of doses of 1 cc. of M/300 sodium cyanide modified the reflex contraction of the tibialis anticus muscle. With progressively increasing rates of elicitation of reflex contraction the changes were: I. Decrease in the reflex amplitude with no change in tonus; II. Decrease in tonus accompanied by an increase in amplitude (occasionally by a decrease or no change in reflex amplitude); III. An undulatory decrease in the tonus which rose and fell with a respiratory rhythm associated with rhythmic changes in the reflex amplitude. Injections of 1 cc. of M/100 sodium cyanide into denervated sinus produced no change in the tibialis anticus reflex.

Extirpation experiments which demonstrate that the neocerebellum is non-essential for any functions previously attributed to the cerebellum. A. D.

KELLER, R. S. ROY (by invitation) and W. P. CHASE (by invitation). Department of Physiology and Pharmacology, University of Alabama.

The demonstration of the non-essentiality of a brachium conjunctivum for the execution of coördinated muscular movement in the monkey raised most prominently the question as to whether the neocerebellum mediates the functions that have been attributed to it. Subsequently (motion picture demonstration this Society 1935, and Physiological Congress 1935) with our attention focused upon postural attitudes the following procedures were accomplished without precipitating any type of dysfunction in the dog: 1, section of the pontile brachia; 2, section of a brachium conjunctivum; 3, complete unilateral removal of the neocerebellar cortex (the whole of the middle lobe of Ingvar, and in addition, the paraflocculus lobule) with sufficient infringement upon the lateral nuclei to degenerate the brachium conjunctivum as evidenced by marchi staining.

The ablation of the neocerebellum unilaterally without precipitating symptoms might indicate bilateral control from the opposite hemisphere. Such is not the case since a subsequent removal of the remaining neocortex has been accomplished without precipitating either crossed or homolateral disturbance.

In the light of the existing view that the cerebellum acts as a whole decortication of the neocerebellum without disturbance might indicate in turn the ability of the anterior and posterior lobes (of Ingvar) to carry the necessary function. If such were the case one would also expect that the lateral lobes would carry the necessary load following removal of the vermis. Such a reciprocal relationship does not exist because removal of the vermis as a whole or the medial nuclei alone leaving the lateral lobes and nuclei intact precipitates typical cerebellar symptoms.

Transitory symptoms which frequently precipitate following lateral lobe removals but which are clearly due to neighboring involvement are: vestibular, leaning and staggering to the contralateral side or actually rotating when there is more extensive involvement; and vermis, staggering to the homolateral side with an overraising of the limbs in walking—which is usually more prominent in the fore leg but at times is more prominent in the rear leg. In the monkey overreaching and postural and spontaneous tremors may appear even in the absence of postural involvements.

Further observations on enhanced appetite with resultant adiposity following removal of the posterior lobe of the hypophysis. A. D. KELLER and W. NOBLE (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

The pars intermedius-infundibular process complex was successfully removed in two dogs without apparent injury to neighboring structures. Dog 499 B exhibited a markedly enhanced appetite both for milk and solid food which continued until a new body weight was reached, by the disposition of fat, after which the food intake returned to normal. Dog 539 B exhibited a typically enhanced appetite for only a few days after operation. It seems probable that a small portion of the pars intermedius (?) remained.

Enhanced appetite with adiposity resulted in Dogs 550 B and 566 B, in which it was attempted to macerate the pars tuberalis leaving the anterior lobe with a view of testing our belief that the pars tuberalis is the source of the antidiuretic fraction of pituitrin. A portion of the pars

tuberalis remained in both instances. In dog 550 B there was a characteristic shrinking of and cellular proliferation in the posterior lobe with intact anterior lobe. Dog 566 B was typical of the dogs described by Cushing where a small portion of the anterior lobe remained and the whole of the posterior lobe was lacking. Adiposity has not occurred under any conditions with total removal of the anterior lobe.

Enhanced appetite with adiposity was also encountered in Dog 520 B in which the hypothalamus was separated from the hypophysis except for a small region in the caudal portion of the infundibulum by a lesion in the ventral tip of the hypothalamus. Characteristic cellular proliferation was present in the infundibular process. It appears however that the infundibular process is not concerned since in Dog 567 B which showed no deviation from the normal the infundibular process showed this same reaction following a small transverse lesion in the hypothalamus, the caudal level of the optic chiasm.

Adiposity and diabetes insipidus which follow hypothalamic lesions are not simply the result of removing nerve fibers which pass to the hypophysis via the infundibulum since a clean separation of the hypophysis from the hypothalamus does not precipitate either of these dysfunctions even transitorially. Diabetes insipidus does however occur if after separation the proximal portion of the hypophysis is destroyed by crushing or cautery.

Motion pictures of dogs and monkeys with various cerebellar involvements. A. D. Keller, R. S. Roy (by invitation) and W. P. Chase (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Demonstration.)

This film will illustrate the findings reported in the paper, "Extirpation experiments which demonstrate that the neocerebellum is non-essential for any functions previously attributed to the cerebellum."

The latency of electric responses in the auditory tracts of the brain stem. E. H. Kemp and G. Coppée (introduced by H. Davis). Department of Physiology, Harvard Medical School.

When concentric electrodes were inserted into the medulla (level of the eighth nerve) of cats under avertin anesthesia, we obtained responses which appeared about 2.3 msec. later than the cochlear response recorded from the round window. This latency decreased from about 2.8 msec. near threshold to 2.2 msec. with an increase of 40 db in the stimulating intensity. With further increase in intensity of stimulation, the latency remained constant. Stimulating frequencies up to 1800 per second were reproduced. Between 1800 and 2700 the responses were largely asynchronous.

When the electrodes were inserted at the level of the lateral lemniscus and inferior colliculus, responses were obtained which appeared about 4 msec. later than the cochlear response recorded at the round window. This latency decreased from about 4.5 msec. near threshold to about 3.7 msec. with an increase of 40 db in the stimulating intensity. With such electrode placements, stimulating frequencies up to 500 per second were reproduced synchronously. Alternation of activity in the individual fibers, shown by a marked decrease in amplitude of the response, began at about 500 per second. Above 1000 per second the responses were completely asynchronous.

We have confirmed the observations of Davis and Derbyshire that responses from the eighth-nerve fibers appear about 1 msec. later than the

cochlear response and that this latency decreases from about 1.3 msec. near threshold to about 0.9 msec. with an increase of 40 db in the stimulating intensity. These eighth-nerve responses were recorded from a wick electrode placed on the round window.

From these facts we infer that only one synapse is interposed between the auditory nerve and the recording position in the medulla, while two synapses are interposed between the auditory nerve and the recording position at the level of the inferior colliculus.

Electrode placements in the medial geniculate body occasionally gave responses which appeared about 5 msec. later than the corresponding wave in the eighth nerve. We attribute our difficulty in obtaining responses from this region to the avertin anesthesia.

Pheo-hemin in plant cells catalyzing respiration. WALTER KEMPNER (introduced by G. S. Eadie). Department of Physiology and Pharmacology, Duke University.

Some ferment reactions which provide the living cells with the required energy are iron-catalyses. Warburg demonstrated this for the respiration of animal cells and many aerobic bacteria. I have shown it for an anaerobic energy supplying reaction: butyric-acid fermentation. Both these iron containing ferments react easily with carbon monoxide like hemoglobin and other organic and inorganic iron compounds. There are, however, many cellular processes insensitive toward CO: lactic-acid fermentation, alcoholic fermentation, respiration of Warburg's so-called yellow ferment, f. e. in bacillus acidificans, in press-juices of yeast and of animal tissues. My question was: Which ferment catalyzes the respiratory mechanism of plants? Is this the chemical function of one of the various hemin compounds found in the plant cells or is plant respiration catalyzed by an iron free substance like the yellow ferment?

I have examined the effect of CO on the respiration of stamens of plum-bloom, stamens and pistils of daffodils, Spanish moss, green pine needles, green leaves of tobacco plants, plum tree and oleander. In all these plant cells I found strong and reversible inhibition of respiration by CO. This proves that the oxygen transferring ferment of respiration in plants is a heavy metal (for CO only reacts at physiological temperatures with heavy metals). The inhibitory effect of CO disappears when the cells are illuminated by strong visible light. This proves that the ferment is a compound of iron (for only iron combinations of CO dissociate in visible light). The sensitivity of the respiration ferment toward CO and the absorption of light by the respiration ferment-CO compound are of the same magnitude in plant cells as in animal cells and aerobic bacteria. These findings indicate that the oxygen transferring ferment of respiration in plant cells is identical with the oxygen transferring ferment pheo-hemin.

Is there a continuous curve of the depth of sleep? N. KLEITMAN, N. R. COOPERMAN (by invitation) and F. J. MULLIN (by invitation). University of Chicago.

Measuring the depth of sleep by the intensity of the stimulus necessary to awaken the sleeper, earlier observers constructed depth of sleep curves. These were usually composite, from data gathered at different hours on several nights, and showed a peak during the first two hours of sleep, then a descent to a low level, with, in some cases, a secondary smaller peak near

the end. On five male subjects we found, by the use of auditory stimuli, that the depth of sleep is related to the motility of the sleeper and depends upon how soon after a movement the subject is stimulated, rather than the hour of the night. After a particular movement the depth of sleep gradually increases, but tends to decrease again as the time for another movement approaches. This creates successive rises and falls in the depth of sleep during the night, and sleep becomes deeper the longer the time interval between two movements. As we previously reported, movements are infrequent during the initial hours of the night's sleep, and in testing for the depth of sleep at random one is more likely to strike periods of deep sleep during these hours than later in the night. Actually, however, with reference to recorded movements of the subject, sleep is no deeper five minutes after a movement at one hour than at another, and, instead of one continuous depth of sleep curve, there are a great number of discontinuous curves between successive pairs of movements, and the maxima of these curves vary directly with the time intervals separating the movements.

The effect of limited cochlear lesions on cochlear potentials and middle ear muscle reflexes. H. KOBRAK, J. R. LINDSAY, H. B. PERLMAN and H. DUBNER (introduced by R. W. Gerard). Departments of Otolaryngology and Physiology, University of Chicago.

In twenty-five rabbits one cochlea was destroyed acutely and the other injured by a small perforation in the apical or middle coil either acutely or, under aseptic conditions, several days before the acute experiment. In all cases middle ear muscle reflexes and cochlear potentials were observed before and after the perforation, except for potentials before chronic lesions.

A sufficiently small lesion did not abolish either potentials or reflex. Larger ones abolished the reflexes entirely while causing no gross change in the electric response. Extensive destruction abolished both. In histological preparations the lower coils of cochleas with an apical lesion, sufficient to abolish reflexes but not potential changes, gave the same picture as entirely normal ones. After moderate injury the potentials were present and reflexes absent for stimuli over a wide frequency range.

In the guinea pig, Bast has shown a persistence of cochlear response after extensive cochlear destruction; whereas Dworkin reports that cats conditioned to a sound stimulus failed to respond after a small cochlear lesion was produced. Our results similarly indicate that the cochlear potential can persist after cochlear injuries which profoundly disturb auditory function, as evidenced by the middle ear reflexes.

Ineffectiveness of parathormone in parathyroidectomized rachitic dogs. F. L. KOZELKA (introduced by A. L. Tatum). Department of Pharmacology, University of Wisconsin.

The effect of parathyroidectomy on rachitic dogs which do not respond to parathormone was studied. The serum calcium reaches a fatal level within ten to twelve hours after the operation. Only a mild tetany is apparent in the forelegs and in no case did it reach the proportions usually observed in normal parathyroidectomized dogs. Three cubic centimeters of parathormone failed to relieve the animals; however, intravenous injections of calcium gluconate gave immediate relief and maintained the animal for a period of six or seven hours. The animals died of respiratory

failure when the serum calcium dropped to 5 mgm. or less per 100 cc. of blood.

A study of the cause of electrical phenomena exhibited in animals and plants. RUTH KROUSE (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

The gastrocnemius muscle of a frog was carefully removed and cut transversely near one end. One non-polarizable electrode was placed against the cut end of the muscle and another against the sound surface. When a delicate galvanometer was connected with the electrodes it indicated a flow of current of 1 to 2 micro-amperes from the sound surface to the injured end of the muscle. The application of a few drops of calcium chloride immediately abolished the electronegativity of the injured end of the muscle and the current of injury while the subsequent application of a weak solution of phosphoric acid or disodium phosphate restored it. That phosphate is present on the freshly injured end of a muscle was demonstrated with the use of ammonium molybdate paper.

It is known that the contracting area of muscle, like the injured, is electronegative to the resting portion. In the area of contraction creatine phosphate and adenylpyrophosphate are hydrolyzed to give rise to the energy for the contraction with the formation of phosphoric acid. The electronegativity of the injured as well as the contracting muscle is attributed to the negative phosphate ions arising from the hydrolysis of creatine phosphate and adenylpyrophosphate.

A green branch was cut from a tomato plant and the cut end placed against one non-polarizable electrode and the sound surface against another. A galvanometer connected in the circuit indicated a flow of current of 1 micro-ampere from the sound surface to the injured end. The application of a weak solution of calcium chloride to the injured end of the branch abolished the electronegativity of the injured end and the demarcation current while the subsequent application of a weak solution of oxalic acid restored it. The electronegativity of the injured portion of the plant is attributed to electronegative oxalate ions. It is known that oxalic acid plays a rôle in plants somewhat similar to that of phosphoric acid in animals.

Histological changes induced in the testes of immature doves and pigeons by gonadotropic hormone. ERNEST L. LAHR (by invitation), OSCAR RIDDLE and ROBERT W. BATES (by invitation). Carnegie Institution, Station for Experimental Evolution.

Use of increase in testis size in the biological assay of follicle-stimulating hormone (F. S. H.) gives importance to a consideration of the structures involved in the rapid growth which accompanies the administration of all extracts rich in that hormone. To the brief description and photographic evidence earlier supplied by Riddle (1931) a more comprehensive account —based in part on more refined preparations—can now be added. Testes stimulated by our numerous F. S. H. + thyreotropic pituitary preparations, by the F. S. H. or by the L. H. preparations of Fevold and Hisaw, by mare's serum, by whole pituitary extract, and by male urine of a subject with embryonal carcinoma of the testis, all show a pronounced growth of the tubular tissue after 4 daily injections. At this stage, intertubular spaces may be more or less evident and intertubular tissue if at all little

increased; these variables appear to depend largely on level of dosage and the individual injected; tubular growth is always evident. More prolonged injections rapidly develop and differentiate the germinal epithelium, and fluid-filled interspaces either disappear completely or become negligible; the intertubular tissues characteristically become less evident. Preparations injected during 10 to 14 days have almost uniformly shown spermatids or spermatozoa, and our evidence indicates that each of the above-named preparations is capable of inducing complete spermatogenesis in a 2-month dove or pigeon testis by 14 daily injections.

Early P. U. preparations containing extremely little of the *A*, and probably much of the *B* factor had little and negligible capacity to increase the weight of the bird testis; with more recent further concentration of the *A* factor their capacity to induce testis growth has increased. The earlier preparations gave slight increase in intertubular tissue; the more recent ones induce definite tubular growth in addition.

Control of micturition by the central nervous system. O. R. Langworthy, L. G. Lewis (by invitation), J. E. Dees (by invitation) and F. H. Hesser (by invitation). Johns Hopkins University.

Injury of certain groups of cells or fibers in the central nervous system produces characteristic changes in vesical activity both in experimental preparations and in patients. These are studied by making records of vesical filling with an air-water system. Individuals with bilateral damage to the cortico-spinal pathway complain of urgency and frequency. They have small bladders due to the fact that the stretch reflex of the vesical muscle is hyperactive. Certain cases with the parkinsonian syndrome also complain of frequency but not of urgency. Fluid is held in the bladder at abnormally high resting pressures, and the capacity is small. With disease of the cerebellum or its pathways the bladder capacity becomes large. The resting intravesical pressure is low. These patients complain of hesitancy, and may develop acute retention. Tabetics have very severe vesical symptoms of hesitancy and incontinence. Graphic records show the characteristic changes found experimentally on cutting the posterior sacral roots. Patients with spina bifida may have urinary symptoms due to trauma of the anterior or posterior sacral roots. If the anterior roots are involved, characteristic, rhythmical, small waves of vesical contraction appear in the record. Both the cortico-spinal fibers and the pathway from the mid-brain controlling tone in the vesical muscle may be injured bilaterally in the spinal cord. Waves of vesical contraction develop which are poorly sustained. Automatic micturition occurs but residual urine is always present.

A study of the conditioned reflex method for producing experimental neurosis. H. S. Liddell, G. F. Sutherland (by invitation), R. Parmenter (by invitation) and T. L. Bayne (by invitation). Cornell University Medical College, Ithaca, N. Y.

Many of the conditions which adversely affect the human nervous system are imponderable factors. On the other hand, the case history of the neurotic animal includes an account of the laboratory situations, measurable and controllable, through which the experimenter has strained the nervous system to the breaking point. In the conditioned reflex laboratory the animal learns to remain quietly on a platform. Limitation of freedom,

first imposed from without, but finally imposed by the animal upon itself, seems to be the fundamental cause of nervous strain, increase of which may later lead to neurosis.

It seems as if the neurotic animal has suffered some special and permanent injury to its nervous system. In fact, in one sheep the neurotic condition persisted until senility at 13 years. The neurotic sheep placed on the platform, when no tests are given, immediately exhibits diffuse and non-adaptive neuro-muscular reactions including frequent and abrupt flexions of the conditioned forelimb. This restlessness, greatest at the beginning of the prolonged period, gradually subsides. The normal sheep, under these conditions, although it stands without motion, shows increasing tension by shallower and more rapid respiration toward the end of the period.

For the purpose of examining the consequences of progressive limitation of freedom during conditioning we selected the pig because its resistance to restraint is violent and varied. Pigs secured food by lifting the lid of a box. At first, complete freedom was allowed and a buzzer signalled delivery of the food. Freedom was gradually restricted by placing the pig on leash, confining it to a small area near the food box, prolonging the intervals between feeding from 30 seconds to 7 minutes, and finally by introducing negative signals. These restraining procedures at first elicited struggles to escape with squealing and defecating, which subsequently gave place to lying or crouching with eyes closed. With habituation to the laboratory routine its activity came to be centered more upon manipulation of the food box and less upon escape. We are now attempting, by such means, to precipitate experimental neurosis in the pig.

The effect of the adrenotropic fraction of the anterior pituitary upon the glycosuria of hypophysectomized-depancreatized and adrenalectomized-depancreatized cats. C. N. H. Long and F. D. W. Lukens (by invitation). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

1. The adrenotropic fractions of the anterior pituitary were kindly donated by Dr. J. B. Collip. They were boiled for ten minutes before use. These extracts did not possess any thyrotropic or ketogenic activity but increased the size of the adrenal cortex of the hypophysectomized rat.

2. When injected into fed or fasted hypophysectomized-depancreatized cats they markedly increased the glucose and nitrogen excretion. This occurred even if the animals were not exhibiting glycosuria prior to the injection. The urine volume was usually doubled.

3. On the assumption that both were derived from the breakdown of protein the increase in nitrogen excretion is sufficient to account for the glucose that appeared.

4. The injection of similar amounts of this extract into adrenalectomized-depancreatized cats did not increase the urine volume, glucose or nitrogen.

The effect of adrenalectomy and hypophysectomy upon the fatty infiltration of the liver following total pancreatectomy in the cat. C. N. H. Long, F. D. W. Lukens (by invitation) and E. G. Fry (by invitation). George S. Cox Research Institute, University of Pennsylvania, Philadelphia. (Read by title.)

Following total pancreatectomy in the cat there develops an intensely

fatty liver. As the following table shows this is largely prevented by removal of all adrenal cortical tissue by hypophysectomy.

	DAYS SINCE PANCREATECTOMY	NUMBER OF ANIMALS	LIVER FATTY ACIDS (GRAMS PER CENT)	
			Average	Range
Normal		9	5.3	2.7–9.0
Depancreatized, adrenals intact	3–7	11	24.4	7.2–48.9
Depancreatised, adrenals denervated	3–8	4	22.6	12.6–30.7
Depancreatized, adrenals demedullated	3–6	5	24.9	14.8–38.9
Hypophysectomized—depancreatized	2–7	6	8.8	3.5–21.0
Hypophysectomized—depancreatized	15–30	3	9.0	3.5–16.3
Hypophysectomized—depancreatized	31–85	5	7.2	2.0–16.8
Adrenalectomized—depancreatized	2–7	5	7.0	3.7–12.0
Adrenalectomized—depancreatized	8–14	6	7.8	2.8–12.8
Adrenalectomized—depancreatized	15–30	4	10.7	3.7–16.1

Adrenalectomized-depancreatized cats. C. N. H. LONG and F. D. W. LUKENS (by invitation). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia. (Demonstration.)

Motion picture records illustrating the striking difference in the behavior of cats depancreatized with and without adrenal cortical tissue. Comparable records will also be shown of hypophysectomized-depancreatized cats, and of cats with all the pancreas, both adrenals and the hypophysis removed.

The excitability of the soma (body and dendrites) of the motoneurones tested with electrical and synaptic stimuli. R. LORENTE DE NÓ (by invitation) and HELEN TREDWAY GRAHAM. Central Institute for the Deaf, and Department of Pharmacology, Washington University Medical School, St. Louis, Missouri.

After arrival of an antidromic impulse (created by a shock delivered to the motor nerve) the excitability of the soma of the motoneurones, tested by synaptic stimuli (impulses set up in nerve fibers ending on the motoneurone) is always decreased throughout an interval lasting as long as 20 msec. But the excitability tested by electrical shocks delivered through bipolar electrodes placed in the neighborhood of the motor nucleus recovers much faster and may become normal after no more than 4 or 5 msec. With this type of stimulation, indications of a supernormal period of excitability have even been found.

The fact that after delivery of an antidromic shock the electrical excitability of the soma of the motoneurones is changed constitutes a proof that the antidromic impulse penetrates into the nerve cell; and the fact that normal or even supernormal excitability to electrical stimuli may coincide with subnormal excitability to synaptic stimuli seems to indicate that the transmission of nerve impulses across synapses is not a process immediately comparable to the stimulation of a nerve cell or fiber by electrical shocks.

A rhythmic series of antidromic impulses reduces the excitability of the soma for synaptic stimuli more than one single antidromic impulse does (summation of subnormality).

A technique for faradic stimulation of tissues beneath the integument in the absence of conductors penetrating the skin. ROGER BROWN LOUCKS (introduced by W. Horsley Gantt). Conditional Reflex Laboratory of the Henry Phipps Psychiatric Clinic, The Johns Hopkins University School of Medicine. (Demonstration.)

In experiments where it is desired to stimulate internal tissues over a period of weeks or months, wires brought out through the skin may prove very troublesome. Fine wires are easily broken. Heavy wires may lead to infection. In 1933 and 1934 the writer published descriptions of a technique which obviates these difficulties. The procedure developed is that of embedding a collodion coated coil beneath the skin and leading insulated wires from the coil to the point to be stimulated. No wires penetrate the integument. This coil, sealed under the skin, will absorb energy from an activated primary "field" coil outside the experimental animal just as the secondary of a transformer absorbs energy from its primary, with which it has no direct wired connection. The current thus induced in the buried coil will be transmitted along the insulated leads to the electrodes at the tissue to be stimulated. An inexpensive but stable thyratron generator, run from commercial power lines, is used to provide the current for the energizing electromagnetic field.

A series of dogs and cats with buried coils will be demonstrated to show the methods of placing electrodes on various neural structures such as cortical sensory and motor projection areas of the brain, subcortical nuclei, and peripheral nerves of the central and autonomic nervous systems.

Further observations on the effect of total adrenalectomy upon experimental pancreatic diabetes. F. D. W. LUKENS (by invitation) and C. N. H. LONG. George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

1. Totally adrenalectomized cats were maintained for one to four weeks in excellent condition by means of cortical extracts. Following pancreatectomy the ensuing diabetes was of a mild nature with moderate glycosuria and absence of ketonuria.

2. Increasing the cortical extract dosage two or three fold above the amount required for maintenance before pancreatectomy did not increase the glucose or acetone body excretion.

3. The carbohydrate tolerance of adrenalectomized-depancreatized or hypophysectomized-depancreatized cats has been examined by a, the response of the blood sugar to injected glucose; b, by the addition of carbohydrate to a protein-fat diet. Both methods reveal that as long as the initial blood sugar is elevated an essentially diabetic response is found. On the other hand, in animals exhibiting hypoglycemia there is an apparent well marked utilization of sugar.

4. Neither thymectomy or thyroidectomy increase the survival nor diminish the glycosuria of totally depancreatized cats.

5. Two cats from which both adrenals, the hypophysis and all the pancreas were removed survived 15 and 21 days respectively. The diabetes of these animals was in no way different from that observed in depancreatized cats with either adrenals or hypophysis excised.

A differential ultraviolet absorption in live and dead cells. B. J. LUYET (introduced by L. V. Heilbrunn). St. Louis University School of Medicine, St. Louis, Mo.

Using the method of ultraviolet photography for the study of absorption in the lower epidermis of the onion bulb scales, the author found that the living and the dead cells are shown respectively (on the negative) by transparent and black spots. This method of distinguishing between live and dead cells checks perfectly with the method of differential staining of Ruzicka. The ultraviolet-absorbing cells can be plasmolysed, the others can not. Killing the cells by various agents—heat, alcohol, etc.—entirely annihilates the absorption power of living protoplasm.

Action potentials of the cerebral cortex and spinal cord before and after cortical stimulation. W. S. McCULLOCH (by invitation) and J. G. DUSSER DE BARENNE. Laboratory of Neurophysiology, Yale University School of Medicine.

Last year we reported that electrical stimulation of the motor cortex extinguished response to appropriately timed subsequent stimulation of the same focus, and demonstrated that extinction was due to local inactivation of that focus, all evidence implicating the large and giant pyramidal cells at the site of stimulation, whereas facilitation appears when the motor cortex is absent.

Now we present cathode ray oscillograms of action potentials of the cortical focus stimulated and of the corresponding spinal region, showing the electrical activity there before and after cortical stimulation.

Two stages of direct coupled amplification, disconnected from the specimen during stimulation, were used with concentric Ag-AgCl-Cl-agar electrodes, the central (a capillary) connected to the grid, the circumferential (4 mm. internal, 6 mm. external diameter), to the cathode. A single pair of these electrodes on the cortex, used alternately for picking up action potentials and for stimulation, gave both pick-up and stimulation maximally nearest to the central electrode.

Cortical action potentials so obtained are *diminished* after extinguishing stimulation. They return very slowly. The more profound and protracted the extinction, the more profound and protracted is the diminution. With no extinction appears no diminution. With the central electrode 5 millimeter in the cord, the circumferential on the dura the spinal action potentials are *augmented* after extinguishing bipolar stimulation of the corresponding cortical focus. They diminish slowly to their original size.

Together these findings confirm the spatial separation of two factors, one for extinction and the other for facilitation, coefficient in determining the size of motor response to second stimulation of a single cortical focus. (Lantern slides.)

The effects of 1-m-hydroxyphenylethanolmethylamine hydrochloride on the bronchial musculature of rabbits and guinea pigs.[1] A. R. McINTYRE. Department of Physiology and Pharmacology, The University of Nebraska College of Medicine, Omaha, Nebraska. (Read by title.)

Changes in lung-lobe volume were recorded in urethane-anesthetized rabbits by the method of Brodie and Dixon (36 experiments) and also by the method of Jackson (6 experiments). The results are essentially the same by either method. 0.25 mgm. of the drug in 1 cc. of Ringer's solution injected into a jugular vein causes a marked rise in general arterial blood

[1] l-m-hydroxyphenylethanolmethylamine hydrochloride is sold under the trade name of Neo-synephrin by Frederick Stearns and Co. to whom we are indebted for supplies of this material.

pressure, but no change in lung-volume. The subsequent injection of 0.1 mgm. of histamine produces contraction of the smooth muscle of the lung as recorded by a diminution of the lung-lobe volume. Relaxation of the bronchial-muscles is not hastened by the injection of as much as 5 mgm. of 1-m-Hydroxyphenylethanolmethylamine hydrochloride, even when injected during the height of the constriction. Doses yielding maximal effects upon blood pressure (2 mgm.) and doses up to 10 mgm. do not prevent histamine-contraction of the bronchial musculature, and are ineffective in causing relaxation when bronchial-contractions are initiated by histamine. The effects of the drug upon blood pressure are similar to those caused by ephedrin.

Very high concentrations of 1-m-hydroxyphenylethanolmethylamine hydrochloride produce a relaxation of the smooth muscle *in vitro* of bronchial-ring preparations from guinea pigs. Its effect is in the order of 1/10,000 that of epinephrin. It is doubtful whether sufficiently high concentrations could be attained *in vivo* in the intact animal with safety to have an effect upon bronchial-muscles.

Ablatory experiments exhibiting the relation between the utricular maculae and the vertical semicircular canals of the frog. W. J. McNALLY (by invitation) and JOHN TAIT. McGill University, Montreal. (Demonstration.)

When the only labyrinthine receptors left behind are the utricular maculae, a (blinded) frog still shows the usual compensatory gravity reactions on a slowly tilted substratum. From absence of its vertical canals it is subject, however, on any attempted movement to heavy bodily pendulation. Furthermore, the animal having only utricular maculae also responds to quick tilt, but the responses thus elicited are anti-compensatory, disturbing balance far more than if the maculae were absent. In (blinded) frogs deprived only of utricular maculae the compensatory adjustments to slow tilt are absent but the vertical canal responses to quick tilt are very marked. When only one single vertical canal, anterior or posterior, is left behind, the precise effect of that canal may be accurately studied.

Some effects of training on the respiratory response to exercise. WALTER C. McNELLY (introduced by F. A. Hitchcock). Departments of Physiology, The Ohio State University, Columbus and Miami University, Oxford, Ohio.

The respiratory exchanges during the performing of a constant amount of work done on a bicycle ergometer of the Prony brake type have been measured in one hundred and twenty-eight experiments using fifty-two human subjects. The Tissot-Haldane technique was used in all experiments. Two spirometers were connected in such a way that consecutive periods could be run without the loss of any expired air. In some experiments Douglas Bags were substituted for spirometers. The experiments fall into three groups: Group I, twenty-six experiments performed on four subjects as controls; Group II, twenty-seven tests on nineteen individuals in training and thirty-three tests on twenty-six individuals not in training and Group III, forty-two tests on three athletes extending over pre-training, training and post-training periods.

The results justify the following conclusions: 1, there is no significant difference in the muscular efficiency of the trained and untrained subjects;

2, the trained subjects had the lower R. Q. and the smaller rise in the quotient during exercise and recovery; 3, the oxygen debt incurred in doing a constant amount of work was greater for the untrained than for the trained subjects and therefore recovery was more rapid with the trained subjects; 4, during exercise the quantity of oxygen absorbed per 100 cc. of air breathed, was greater for the trained than the untrained subjects. This is true in spite of the fact that during the preliminary rest period there is no significant difference between this figure for the trained and the untrained subjects.

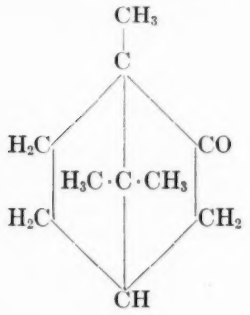
Comparison of cobra venom and morphine as analgesics. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

The writer has published elsewhere experimental and clinical data proving the analgesic property of cobra venom when injected in small doses (Proc. Natl. Acad. Sciences, January, 1936). It was demonstrated that this action of cobra venom, like that of morphine, has its seat in the brain. In relieving pain, however, cobra venom is much slower in onset than morphine and longer in duration. This difference is partially explained by the present findings obtained in experiments on auto-oxidative processes in brain and muscle tissue. Studies *in vitro* were made on oxydases of fresh muscle and brain tissue from rats, guinea pigs and cats by Thunberg's method of reducing a buffered solution of methylthionine chloride in vacuum tubes. Both brain and muscle suspensions treated for half an hour with morphine sulphate (10 mgm. to 1 cc. of suspension) revealed definite inhibition of enzymatic activity. Longer exposures to morphine produced less depression; and after ten hours' exposure no inhibitory effect could be detected, a finding indicating a chemical change in the alkaloid. In similar experiments with cobra venom little change was effected in one hour by exposure to action of 5 mouse units of venom, a moderate therapeutic dose; but when the period of exposure was prolonged for four hours or more, marked inhibition of enzymatic activity was produced. Experiments were also made *in vivo* by injecting cobra venom and morphine into animals and killing them after the drugs had taken effect. In such experiments cobra venom markedly retarded oxidation of both brain and muscle tissue suspensions, indicating a relatively greater localization of the drug in the brain. When morphine was thus injected, however, it was found that the alkaloid was rapidly destroyed by both brain and muscle, and more rapidly by the brain. It appears, therefore, that cobra venom remains unchanged in the brain longer than morphine, which at least partly explains the difference in duration of analgesia effected by the two drugs.

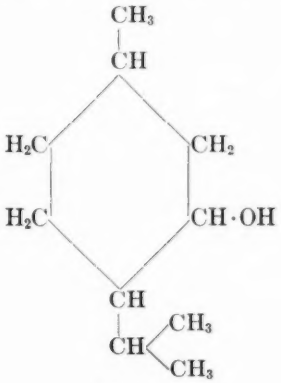
Comparative physiological activity of camphor and menthol. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Several specimens of menthol, natural (U. S. P.) and synthetic, were compared with ordinary camphor (U. S. P.) in respect to their action on living animals and plants. Dilute hydro-alcoholic solutions were tested on seedlings of *Lupinus albus* and injected intravenously in cats under light ether anesthesia to ascertain their effect on circulation and respiration. Solutions in olive oil, 2 per cent, were injected intraperitoneally in mice.

Menthols, in concentrations of 1:10,000 to 1:5,000, inhibited root growth of *Lupinus* seedlings 25 and 50 per cent, respectively, and were more toxic than camphor in the same concentrations. Twenty-milligram doses of oil solutions, injected in mice, produced unconsciousness and depression of respiration, lasting for six hours or more, from which they gradually recovered. When administered in the same quantities, camphor produced



Camphor



Menthol

no unconsciousness but violent clonic and tonic convulsions. The lethal dose of menthol for mice, weighing from 20 to 22 grams, was 40 mgm., a dose which killed within twelve hours. The same quantity of camphor produced death in from two to three days. Twenty-milligram doses of menthol, intravenously injected, markedly depressed the circulation and respiration of cats, while camphor in similar doses had little effect. There was no significant difference in toxicity between natural and synthetic menthols.

Influence of opium alkaloids and cocaine on auto-oxidation of brain and muscle tissues. DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Suspensions of fresh muscle and of brain tissues from rats, guinea pigs and cats were studied by Thunberg's method of reducing a buffered methylene blue solution in specially constructed vacuum tubes. The oxidative reactions of normal controls were compared with those of tissue emulsions exposed for different intervals of time to morphine sulphate, codeine phosphate, thebaine hydrochloride, papaverine hydrochloride, pantopon and cocaine hydrochloride, respectively. Ten milligrams of morphine sulphate added to 1 cc. of muscle suspension for from thirty to sixty minutes produced a marked inhibition of enzymatic activity, as demonstrated by rapidity of decolorization of the dye. Codeine, or methyl morphine, was also depressant but less so than morphine alone; and thebaine, or dimethyl morphine, was less depressant than morphine. Longer exposures (for ten hours or more) to morphine were less effective than short

exposures, thus indicating a chemical change in the alkaloid. Papaverine, the only member of the isoquinolin group of alkaloids to be studied, was much more depressant than any member of the phenanthrene series. Pantopon was more depressant than morphine, possibly because of the synergism effected by the papaverine present. Cocaine was very depressant for the oxidative enzymes. Similar findings were made with brain tissue emulsions but the inhibitory effects of morphine were of short duration so that when the drug remained in contact with the brain tissue for over an hour, a chemical change took place and morphine no longer exerted an inhibitory action on the oxidative processes but actually accelerated the reduction of methylene blue.

Effect of deuterium oxide on action of some enzymes. DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

In connection with a study of certain animal and vegetable enzymes, it was found that deuterium oxide, or heavy water, and ordinary H_2O, respectively, exhibited a striking difference in their physiological action. The enzymes studied were *oxydases* of fresh rat muscle, rat brain and cat brain tissue, and the reductase of finely ground *Lupinus albus* seeds. Suspensions of the animal tissues were made in physiological NaCl solutions prepared with D_2O and H_2O, respectively. The *Lupinus* seeds were extracted with plain D_2O and H_2O without salt. The writers employed Thunberg's method of determining the rapidity with which a standard buffered solution of methylene blue is decolorized in specially constructed vacuum tubes. A distinct difference in velocity of enzymatic activity was discovered. Concentrations of D_2O as low as 1:2,000 were observed to *accelerate* the reduction of the dye to the leuko form. A diphasic effect was sometimes obtained with stronger concentrations of D_2O, such as 1:100—a primary slowing of the enzymatic action followed by a more marked acceleration. Similar differences in the physiological action of D_2O and H_2O were found in experiments with *catalase* of muscle, *brain* and vegetable tissues. The findings described above are corroborated by the recent studies of Fox and Craig on hydrolysis of starch (Proc. Soc. Exper. Biol. and Med. **33:** 266, 1935).

Antagonistic action of follicular and corpus luteum extracts on bitterlings. DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Experiments were made on bitterlings (*Rhodeus amarus*) according to the method described by Kanter, Rauer and Klawans (J. A. M. A. **103:** 2026, 1934). Preparations of the follicular hormone, placed in water containing the bitterlings produced marked elongation of the ovipositors in from 24 to 48 hours. Corpus luteum extracts prepared by a method described elsewhere (Proc. Soc. Exper. Biol. and Med. **27:** 152, 1929) produced little or no stimulation of the ovipositors. In a third series of experiments, when the follicular and corpus luteum extracts were added simultaneously in suitable proportions, a definite inhibition of the stimulating action of the follicular hormone was observed. It was found that extracts obtained from diseased corpora lutea were not as effective in this

respect as those derived from normal glands. These experiments indicate an antagonism between the corpus luteum and follicular or estrogenic hormones. To obtain reliable results in such experiments, it is necessary to use fish which have previously given a positive reaction to the estrogenic hormone and been allowed to recover in a two- or three-week interval because some bitterlings do not respond effectively to that hormone because of immaturity or stunted growth.

Physiological action of some N-methyl amides. DAVID I. MACHT and GAETANO F. D'ALELIO (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., and Laboratory for Organic Chemistry, Johns Hopkins University, Baltimore, Md. (Read by title.)

The writers synthesized a series of nine compounds with a general formula of $R \cdot CO \cdot NH \cdot CH_3$, the first member having one hydrogen atom in place of R and the higher ones, alkyl groups ranging from CH_3 to C_8H_{17}. Aqueous and weak hydro-alcoholic solutions were studied pharmacologically 1, on seedlings of *Lupinus albus;* 2, on larvae of *Rana sylvatica;* 3, on goldfish; 4, on white mice, intraperitoneally injected; 5, on circulation and respiration of cats, intravenously injected; 6, on surviving smooth muscle tissue, and 7, on rabbit's eye and frogskin for local anesthetic effect. The toxicity of the compounds for plants and goldfish increased with their molecular weight. Doses of from 5 to 20 mgm. of the N-methyl amides, injected in mice, led rapidly to unconsciousness, paralysis of the respiration and death. Doses of from 10 to 20 mgm. in 10 per cent alcohol, intravenously injected in cats kept under light ether anesthesia, were markedly depressant for blood pressure and respiration, the toxicity of the compounds increasing with their molecular weight. The first five members of the series had little or no effect on surviving smooth muscle. The higher members were depressant. Compounds VII, VIII and IX, in 5 per cent alcohol, were definitely anesthetic locally, the highest member being the most potent in this respect.

Physiological action of some N-ethanol amides. DAVID I. MACHT and GAETANO F. D'ALELIO (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., and Laboratory for Organic Chemistry, Johns Hopkins University, Baltimore, Md. (Read by title.)

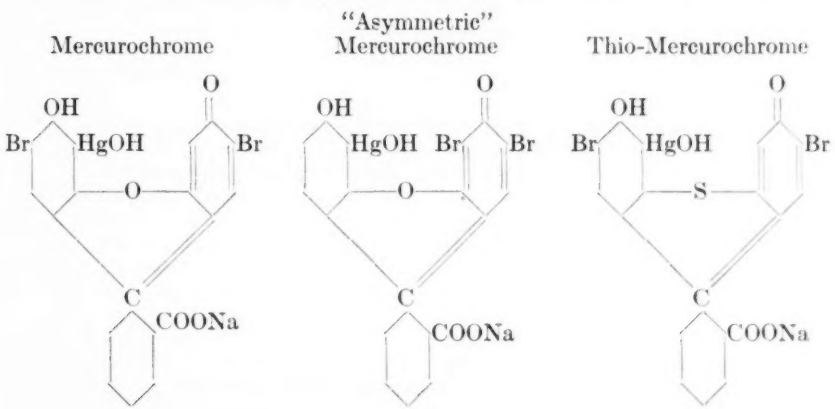
The writers synthesized a series of N-ethanol amides, corresponding to the N-methyl amides, and studied their physiological properties by the methods described above. The general structure of this series is represented by the formula, $R \cdot CO \cdot NH \cdot CH_2CH_2 \cdot OH$, R being substituted by hydrogen in the first member and by alkyl radicles in the succeeding members of the series. Tests were made on seedlings, on tadpoles, on goldfish, on smooth muscle tissues, on mice, on the blood pressure and respiration of cats and on the rabbit's eye for local anesthesia. The N-ethanol series was less toxic than the N-methyl amide series. No local anesthesia was produced by any member of this series. The toxicity of the different members, with one exception, increased with the molecular weight. Compound VI (C_5H_{11}) was found to be more toxic than compound VII (C_6H_{13}). This exception emphasizes the danger of making any sweeping generalization with regard to relationships between chemical structure and physiological action which is not experimentally verified.

Responses to drugs of Ursus americanus. DAVID I. MACHT and MARY ELEANOR DAVIS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Studies were made of the responses to pharmacological agents of a small black bear weighing approximately 20 kilo, which was obtained from the Dismal Swamp. After its reactions and behavior had thus been observed for four weeks in the laboratory, the bear was anesthetized; and studies were made under ether on its respiration, circulation, kidney and liver function. Finally, surviving tissues from its intestines, bladder, vasa deferentia, arteries, etc., were tested pharmacologically *in vitro*. The drugs tested altogether *in vivo* and *in vitro* were atropine, homatropine, pilocarpine, physostigmine, epinephrine, ephedrine, pituitrin, nitroglycerin, amyl nitrite, phenolsulphonphthalein, bromsulphalein, Bromsalizol, croton oil, morphine, apomorphine, papaverine, pantopon, phenobarbital and ether. Most of the drugs elicited the same responses in the bear that they produce in the dog and rabbit. The derivatives of opium were the chief exception. Even in repeated doses of 65 mgm., apomorphine, given by mouth, produced no vomiting. Morphine, injected in doses of less than 100 mgm., had little effect. Larger doses of both morphine and pantopon produced marked excitement followed by mild sedation with mydriasis instead of contraction of the pupils. To quiet the animal sufficiently to permit anesthetization, the writers were obliged to administer in its food 150 mgm. of morphine sulphate, 200 mgm. of pantopon, and 0.7 gram of luminal in two doses. When the experiments had been concluded, the animal was rapidly dispatched by applying 1 small drop (0.05 cc.) of nicotine to its tongue.

Physiological action of asymmetric mercurochrome and thio-mercurochrome. DAVID I. MACHT and WILTON C. HARDEN (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Ordinary mercurochrome is oxy-mercury-di-brom fluorescein with two bromine atoms arranged symmetrically as in the first of the three formulae.



An asymmetric, or unsymmetrical, mercurochrome in respect to bromine atoms was prepared by fusing di-brom-para-hydroxy benzoyl benzoic acid with resorcin, which on mercurating yields a compound represented by the second formula. A third mercurochrome was prepared from thio-fluorescein which yielded a mercurochrome with a sulphur atom such as is shown in the third formula. These compounds were compared with normal mercurochrome in experiments on rabbits and living seedlings of *Lupinus albus*. There was found to be no difference in their toxicity for rabbits either in respect to their effect on kidney function or in respect to their lethal dosage, which averaged about 25 mg. per kilo. When tested on *Lupinus* seedlings, asymmetric mercurochrome solutions (1:10,000 to 1:5,000) proved to be definitely less toxic than normal or symmetrical mercurochrome. Thus, by phytopharmacological tests it is possible to differentiate between ordinary and asymmetric mercurochromes. The bactericidal properties of all these varieties of mercurochrome were found to be the same. Thio-mercurochrome acted like ordinary mercurochrome.

The rôle of tonic reflexes in the postural reactions to cerebellar stimulation. H. W. MAGOUN (by invitation). Northwestern University Medical School.

Stimulation of the medial portion of the interior of the cerebellum in the cat yields a biphasic postural response. The typical rebound or second phase of this reaction, obtained at the cessation of a stimulus, is a prolonged contraction of the extensors of the ipsilateral limbs, the flexors of the contralateral limbs, and the contralateral axial muscles. All of these contractions are inhibited during stimulation. These rebound contractions commonly persist for five minutes or longer following brief stimulation of the cerebellum in the normal animal, and the question arises as to what activity is responsible for the long-lasting discharge of efferent centers which underlies the maintenance of these postures.

The rôle of tonic reflexes in the prolonged maintenance of these rebound postures was studied by observing the duration of rebound contraction after the elimination of tonic reflexes. These observations were made on a deafferented forelimb after fixation of the neck in the midline, denervation of the opposite forelimb, and elimination of the hind limbs by section of the lower thoracic spinal cord. In six of the thirteen cats used, bilateral labyrinthectomy was also performed. Deafferentation and labyrinthectomy were chronic, the other operative procedures were performed at the time of experiment.

It was found that the duration of the prolonged rebound contraction which follows the cessation of stimulation of the cerebellum in the normal animal, was considerably reduced by deafferentation of the reacting limb, and appeared to be decreased a little more by labyrinthectomy in addition to deafferentation. But after the elimination of all of the afferent impulses upon which the tonic effects of the stretch and labyrinthine reflexes depend, continued discharge of both flexor and extensor centers for the forelimb was observed for periods varying from a few seconds up to a minute or longer.

The hypophysectomized chimpanzee. WILLIAM MAHONEY (introduced by J. F. Fulton). Laboratory of Physiology, Yale University, New Haven. (Read by title.)

Hypoglycemia hypyphysiopriva in the lower animals has been observed

and reported by various workers. The higher animals likewise are susceptible, and the following experience with a chimpanzee is characteristic of observations made upon other hypophysectomized primates (*Macaca mulatta, Cercocebus torquatus atys, Papio papio*).

The pituitary body of a healthy male chimpanzee aged 6 years, weighing 18 kilograms, was completely removed by the subtemporal route. The animal made a good recovery and ate normally for one and a half days. On the third day he was found unconscious, presumably from hypoglycemia, and was given 25 grams of 5 per cent glucose intraperitoneally. After 15 minutes he grasped objects, and in 45 minutes he responded to his name and drank water; he walked about the cage in 2 hours. He continually refused food so it was necessary to feed him by stomach tube for one week before he ate normally. No polyuria or polydipsia occurred. Three weeks after operation the animal was in good condition, and, as was done before operation, was given sodium amytal anesthesia to test sugar tolerance. On each occasion he was fasted for 12 hours, then received intravenously 1 gram of dextrose per kilogram of body weight. Blood sugars (Hagedorn-Jensen) were done fasting, then 15, 30, 60, 120, and 180 minutes after dextrose was given. The preoperative curve in milligrams per cent was: 70, 280, 230, 142, 86, 85, and after hypophysectomy: 43, 217, 149, 45, 22, 18. Death occurred as a result of inhalation of vomitus while under anaesthetic 17 days after operation. Autopsy revealed no pituitary tissue. The hypothalamus was studied in serial section with the Nissl technique and no recognizable retrograde cell changes were found in any of the hypothalamic nuclei (supraoptic, paraventricular, tuber, mammilloinfundibular, ganglion basale of Meynert). The superior cervical sympathetic ganglion also did not show retrograde reaction.

Comparative effects of oral administration of sucrose, fructose and glucose to phlorhizinized dogs. R. S. Manly and W. R. Murlin (introduced by John R. Murlin). Department of Vital Economics, University of Rochester, Rochester, N. Y.

Determinations of sugar and ketone substances in blood, and sugar, nitrogen and ketone substances in urine, were made on phlorhizinized dogs maintained on a diet of ground beef. The comparative effects of oral administration of 25 to 50 gm. of sucrose, fructose and glucose were studied.

The sugars arranged in order of their nitrogen sparing effect are as follows: fructose > sucrose > glucose. The increase in urinary sugar was more rapid after administration of glucose than after the other sugars. On the average, the rise in blood sugar following glucose was twice that obtained after feeding fructose or sucrose; the rise in blood sugar, however, was maintained for a longer period with fructose and sucrose than with glucose.

Although the three sugars exerted somewhat differing effects on ketonemia and ketonuria, the ketogenesis or actual ketone substance production in the blood-urine system was lowered equally by all three sugars. In some cases the carbohydrate apparently destroyed already formed ketone substances. A simple formula for the calculation of ketogenesis has been developed.

The effect of sulfur on rate of respiration and on the respiratory quotient in Chilomonas paramecium. S. O. Mast, D. M. Pace (by invitation) and

LOUISE R. MAST (by invitation). Zoological Laboratory of The Johns Hopkins University. (Read by title.)

Chilomonas (a colorless animal) grows well in water which contains $MgSO_4$, NHCl, K_2HPO_4, and $NaC_2H_3O_2$ and in this solution minus acetate if the carbon dioxide pressure of the air is increased and silicon is added. It can without chlorophyl or light, obtain nitrogen from ammonia and carbon from acetate or carbon dioxide.

The specimens grown in this solution contain much starch and little fat and the consumption of oxygen at 24° is 0.17 ± mm^3 per 10000 individuals per hour.

If sulfur is omitted starch remains nearly constant in quantity but fat rapidly accumulates until it results in death (fatty degeneration). The consumption of oxygen remains nearly constant for about six hours and then decreases from 0.17± to 0.06 ± mm^3/10000/hr. during the next 14 days.

If acetate is omitted, the chilomonads decrease greatly in size; starch and fat decrease to zero in about three days and the consumption of oxygen decreases from 0.17± to 0.07 ± mm^3/10000/hr. in 24 hours.

If sulfur and acetate are omitted, starch decreases to zero and fat accumulates until it results in death, and the consumption of oxygen decreases from 0.17± to 0.02 ± mm^3/10000/hr. in 24 hours.

Starch changes to fat.

Sulfur induces oxidation of fat and thus facilitates respiration and prevents accumulation of fat.

Oxidation of fat is probably associated with reduction of *cystine* to *cysteine*.

Values for the respiratory quotient were obtained for *Chilomonas* in solutions with and without sulfur, acetate, sugar and amino acids respectively, and for *Paramecium* in spring water.

There is no correlation between the values obtained and the composition of the solutions used.

The total average of the values obtained for the respiratory quotient is 0.31± for *Chilomonas* and 0.71± for *Paramecium*.

The relatively low values obtained for *Chilomonas* indicates that in all the solutions used some of the carbon dioxide produced by it is consumed in synthesis of organic compounds, i.e., that even in solutions containing acetate, sugar and amino acids it obtains some carbon from carbon dioxide.

The effect of silicon on growth and respiration in Chilomonas paramecium.

S. O. MAST and D. M. PACE (by invitation). Zoological Laboratory of The Johns Hopkins University. (Read by title.)

In solution SO_4 which consists of NH_4Cl, K_2HPO_4, $MgSO_4$, $NaC_2H_3O_2$ and H_2O, *Chilomonas paramecium* divides 3.5± times per day at 24±° and lives indefinitely.

If silicon in optimum concentration is added to solution SO_4 the rate of growth and the frequency of division increases more than 26 per cent.

The optimum concentration of silicon in solution SO_4 is 0.000016 M or possibly somewhat lower.

If sulfur is omitted from solution SO_4, *Chilomonas* divides only a few times and dies out in 3± days. If sulfur is omitted and silicon added in optimum concentration, it divides freely for a few days and lives 6± days.

Silicon in part takes the place of sulfur.

In solution SO_4 the rate of consumption of oxygen at 25 ± 0.01°C. is 0.263 ± mm^3 per hour per 10000 chilomonads.

If silicon in optimum concentration is added to solution SO_4, the rate of respiration increases nearly 24 per cent.

If silicon is added to solution SO_4 with sulfur omitted, there is but little if any increase in rate of respiration. Oxidation in chilomonads depends largely if not entirely upon sulfur.

If silicon is added to solution SO_4 with acetate omitted, there is marked increase in the rate of respiration for 5± hours and no increase after that. The increase in rate of respiration during the first 5± hours is probably due to acetate which was in the chilomonads when they were transferred from the culture solution to the solution without acetate.

The effect of silicon on the rate of respiration is due largely if not entirely to its catalytic action on synthesis of complex organic compounds which are oxidized by the action of sulfur.

All the results presented above were obtained in experiments on chilomonads in sterile solutions.

Effects of cyclopropane anesthesia on the heart. WALTER J. MEEK and PERRY P. VOLPITTO (by invitation). University of Wisconsin, Madison. (Read by title.)

We have previously shown (J. Pharmacol. Exper. Therap. **51:** 1, 1934) that cardiac arrhythmias often occur under certain concentrations of cyclopropane. Although the rapid disappearance of these irregularities on lightening the anesthesia would indicate that no permanent cardiac damage had resulted, this point has been further investigated. Dogs, decerebrated under ether, were tested relative to the effect of variations in venous pressure on cardiac dilatation. Intravenous injections of salt solution were used to vary venous pressure and cardiac dilatation was estimated from x-ray silhouettes. The animals were then anesthetized with known concentrations of cyclopropane and the effects of venous pressure in distending the part again determined, the argument being that if the myocardium were weakened a greater distention for a given venous pressure would be brought about. Ordinary variations in heart rate do not affect this response. Myocardial injury from chloroform can readily be shown by this method. In six dogs under 20 to 30 per cent cyclopropane only one showed any dilatation of the heart and in this case the anesthesia was carried down to respiratory arrest several times. It is concluded therefore that cyclopropane in the usual anaesthetic range is not likely to injure the heart, at least as tested by the method used.

The influence of diet upon the regeneration of serum protein. DANIEL MELNICK (by invitation), GEORGE R. COWGILL and ETHEL BURACK (by invitation). Department of Physiological Chemistry, Yale University, New Haven, Connecticut.

Using as a "constant and maximal stimulus" for regeneration of serum protein the maintenance of its level at about 4 per cent by plasmapheresis of varying degree, we have studied the effect of single proteins in artificial "synthetic" diets in promoting the regeneration of serum protein. We have endeavored to quantitize all factors involved. Our technique permits us *a*, to bleed quantitatively to within ±1 per cent of the calculated amount so that the stimulus for regeneration is constant from day to day;

b, to deplete the dogs to the basal level (3.5 to 4.2 per cent) within one week by the use of our artificial protein-free diet with simultaneous plasmapheresis during which the animals are bled $\frac{1}{4}$ of their blood volumes daily; *c*, by our type of experimental diet to know and control the intake of all nutrients and calories so that protein is the only dietary variable operating, and *d*, to correct for that fraction of dietary protein required for nitrogen equilibrium, by determining previously for each tested protein the minimum needed for nitrogen balance.

The ratio of the amount of serum protein per week removed by bleeding above that regenerated by the animal when eating the basal protein-free diet, to the dietary protein increment (i.e., above that required for nitrogen equilibrium) is termed the potency ratio. The following values were obtained for serum protein, lactalbumin and casein respectively: with dog 3, 0.53, 0.38 and 0.45, and dog 4, 0.64, 0.34 and 0.42. Dog 3 was able to regenerate 13.4 grams serum protein per week while on the protein-free diet (five determinations) and dog 4, 9.6 grams (four determinations).

Dog 5, subsisting on the protein-free diet but with plasmapheresis discontinued, exhibited inhibition in its return to normal serum protein level, yielding values between 4.0 and 5.2 per cent during 45 days; fasting (18 days) resulted, after a slight delay, in a progressive rise in the serum protein level to 6.2 per cent, the original "normal" being 6.75 per cent. An interrelationship was observed between the blood volume, hematocrit and serum protein level.

The source of blood acetone and the site of the antiketogenic action of insulin.

I. Arthur Mirsky (introduced by Samuel Soskin). Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati, O.

The production of acetone bodies generally has been ascribed to a disturbance in the relative proportions of fat and carbohydrate that are being coincidentaly oxidized by the tissues. Thus, it has been assumed that whenever there is a decrease in available carbohydrate (ketogenic diet or starvation) or in such conditions as phlorhizin and pancreatic diabetes, the oxidation of carbohydrate is decreased and the oxidation of fat is correspondingly accelerated, the acetone bodies arising in consequence of an incomplete oxidation of the latter.

There is considerable evidence in the literature which indicates that the muscles can account for very little, if any, of the acetone bodies which accumulate in the blood. It has also been shown that in all the above conditions the muscles continue to use carbohydrate. In view of these contradictions, it seemed of interest to investigate a ketosis produced by means other than the production of diabetes or carbohydrate starvation.

We utilized an extract of the anterior lobe of the hypophysis which has been shown, by a number of investigators, to be ketogenic in action. The administration of our extract to normal rabbits caused the immediate appearance of acetone bodies in the blood, to average values of 14 mgm. per cent in the first hour, 18 mgm. per cent in the second hour, and 14 mgm. per cent in the third hour. However, in no instance was acetone found in the blood of liverless rabbits, even after relatively larger doses of the extract. Furthermore, the simultaneous administration of insulin with the ketogenic principle to normal rabbits almost completely inhibited the ketonemic response.

These results demonstrate that the ketogenic principle of the anterior lobe of the hypophysis acts solely on the liver and that the muscles make no significant contribution to the ketosis. They further indicate that, if a relative decrease in carbohydrate oxidation is a factor in ketone formation, this condition may apply to the liver but not necessarily to the muscles. Finally, the antiketogenic action of the insulin shows that, for this important function at least, the site of action of insulin is the liver and not the muscles.

A bloodless method for continuous recording of peripheral circulatory changes. HANS MOLITOR and MICHAEL KNIAZUK (by invitation). Merck Institute of Therapeutic Research, Rahway, N. J. (Demonstration.)

The method is based on the principle of simultaneous recording of skin temperature and light transmission through the skin. The amount of light transmitted varies with the amount of blood circulating through the observed area. By using a light source of constant intensity and measuring the amount of transmitted light by a photocell, the current output of this cell is in direct relationship to the blood supply. In order to differentiate between active hyperemia and congestion, the surface temperature as well as light transmission are simultaneously measured and recorded. During active hyperemia the skin temperature rises and the amount of transmitted light decreases. During congestion the skin temperature remains unchanged or decreases, while the amount of transmitted light decreases.

Influence of the forebrain on the reflex responses of the nictitating membrane of the cat. R. S. MORISON (by invitation) and D. MCK. RIOCH. Departments of Physiology and Anatomy in the Harvard Medical School.

The responses of the nictating membrane to a standard central stimulation of the sciatic nerve in cats under urethane with the vagi cut and carotid sinuses denervated had the following characteristics: a, great variability in the rate of rise and total height; b, strong positive rebound; c, prolonged after-discharge. Destruction of the cortex anterior to the cruciate sulcus resulted in a decrease of the rebound and after-discharge without diminution of the height of the total responses. Removal of the remaining cortex gave no further change. Transection caudal to the hypothalamus was followed by considerably smaller responses showing little variability, shorter after-discharge, and no rebound.

Stimulation of the sigmoid gyri along the margins of the cruciate sulcus and of the internal capsular fibers coming from this region facilitated the reflex responses. Stimulation directly rostral to the anterior sigmoid and of the associated internal capsule gave marked inhibition. These effects were still obtainable after removal of the neopallium caudal to the posterior sigmoid gyrus. In two preparations with right neopallium removed six months previously, moderate facilitation was obtained from the tuberculum olfactorium, the pyriform lobe, the hippocampus and the fornix, and marked facilitation from the lateral hypothalamic area, the posterior hypothalamus, and the region of the nucleus hypothalamicus.

The effect of the cardiac sympathetics and adrenalin upon ventricular rhythms induced in cats by inhalation of petroleum ether. L. H. NAHUM, E. C.

HOFF and H. E. HOFF. Laboratory of Physiology, Yale University, New Haven.

Dautrebande has shown that inhalation of petroleum ether causes peripheral vascular paralysis similar to that induced by benzol. Since benzol in association with adrenalin and intact cardiac sympathetics causes grave alterations in cardiac rhythm, the following experiments were carried out to determine electrocardiographically the effect of petroleum ether upon the heart.

Two types of experiments were performed upon cats: a. In one, the animals were under amytal. During deep anesthesia, inhalation of petroleum ether rarely produced ventricular extrasystoles. These however readily appeared if 0.5 cc. of adrenalin was administered just before or immediately after inhalation. This amount of adrenalin alone never caused extrasystoles. b. In the second type of experiment, petroleum ether was inhaled by high decerebrated cats. In such animals, the cardiac sympathetics are in a high state of activity (Beattie, Brow and Long; Hering). The first effect of inhalation was a slowing of the heart which was soon followed by frequent ventricular extrasystoles from several foci. Only rarely was ventricular tachycardia encountered. As the inhalation progressed the abnormal rhythms tended to subside but reappeared during the recovery period. Four to five hours after decerebration, inhalation of petroleum ether no longer produced ventricular extrasystoles, but they promptly appeared if 0.5 cc. of adrenalin was injected a few minutes before or immediately after inhalation.

Petroleum ether therefore is of a lower order of toxicity than benzol in its effect upon the heart, since the ventricular rhythms occur only in animals with a high degree of cardiac sympathetic activity or a high adrenalin content of the blood.

The distribution of a chemical excitant for the glands of the jejunum. E. S. NASSET. Department of Vital Economics, University of Rochester, Rochester, N. Y.

In an earlier paper (Nasset and Pierce, Am. J. Physiol. **113:** 568, 1935) it was demonstrated that an acid alcohol extract of small intestine from both dogs and swine contained a substance which excited the glands of the jejunum to secrete.

Extracts were made of various tissues from six different animals, namely: dog, cat, hog, man, cow and rabbit. The extract of small intestine of all species except rabbit was potent. Stomach extracts were inactive. The bovine stomach and colon were not extracted. With the exception of rabbit, the colon extracts were quite active and contained little or no secretion. One batch of cat colon extract increased the secretion of the jejunum without affecting the pancreas and with only a slight fall in blood pressure. Extracts of liver, spleen, pancreas, kidney and skeletal muscle were uniformly inactive. Since the assays were all made on acute dog preparations it appears that the active substance is not species specific and that it is found in appreciable amounts only in the small intestine and colon.

The reaction of the dog's stomach to acetylcholine. H. NECHELES, P. LEVITSKY (by invitation), R. KOHN (by invitation) and M. MASKIN (by invitation). Department of Gastro-Intestinal Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

After it had been found that the venous outflow from the rat's stomach is greatly reduced by acetylcholine (Proc. Am. Physiol. Soc. 1935), the effect of this drug on the dog's stomach was tested with various methods:

1. Perfusion of isolated arteries and veins. Vasoconstriction was obtained—the veins contracted to a greater extent than the arteries.

2. Viviperfusion of the excised stomach of one dog through the carotid artery of another dog (return flow through the external jugular vein). Small doses of acetylcholine (1 gamma) injected through the hepatic artery, produced diminished venous outflow in the majority of instances, while larger doses resulted in increased venous outflow.

3. Measurements of the venous outflow from the innervated stomach in situ, during the injection of acetylcholine into the arterial side (splenic artery) confirmed the results obtained in the extirpated and viviperfused stomachs on the whole, but in some experiments only diminished outflow could be obtained.

4. Oncometry of the extirpated and viviperfused stomach revealed increase of stomach volume when acetylcholine was injected into the arterial side (hepatic artery).

From the above experiments it seems that acetylcholine in small doses produces vasoconstriction in the dog's atomach. This factor may play a rôle in the pathogenesis of peptic ulcer, since it is known that acetylcholine is released in the wall of the stomach, under vagus stimulation.

A comparison of the effect of different anesthetics on the electrical potential of the cerebral cortex. H. W. Neild (by invitation), W. P. Elhardt (by invitation), G. C. Wickwire (by invitation), O. S. Orth (by invitation) and W. E. Burge. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

The effect on the electrical potential of the cortex of the cerebrum of nitrous oxide, ethylene, ether, and chloroform was determined in the following way. The sciatic nerve of a dog, anesthetized with ether, was exposed, as well as a portion of the cerebral cortex, by the removal of a piece of the cranium and the meninges. One non-polarizable electrode was placed against the cortex of the brain and another against the sciatic nerve. A delicate galvanometer was connected with the electrodes.

The cortex of the brain of dogs slightly anesthetized with either nitrous oxide, ethylene, ether, or chloroform was found to be electronegative and the sciatic nerve electropositive and the galvanometer indicated a flow of current from the positive sciatic nerve to the negative cortex of the brain of two to four micro-amperes. An increase in the depth of anesthesia by all four of these anesthetics brought about a decrease in the negative potential of the cortex of the brain from two or three micro-amperes to less than one micro-ampere but only ether caused a reversal in polarity, that is, caused the cortex to become electropositive and the sciatic nerve electronegative.

It was found difficult with the use of any of these anesthetics to decrease the negative potential of the cerebral cortex of a dog partially intoxicated with ethyl alcohol in keeping with the fact that anesthetization of such an animal is difficult. However, large intoxicating doses of alcohol like the other anesthetics was found to decrease the negative potential of the cortex of the cerebrum and in fact, to produce a reversal in polarity.

The electrical potential of the brain could be more easily and extensively

changed and controlled with ether than with any of the other anesthetics and in this respect, ether may be considered the best of the four general anesthetics used. Most trouble was encountered with chloroform, the anesthetic that was expected to produce the greatest decrease in the negative potential of the cerebral cortex. It produced a significant decrease in the negative potential, sufficient to induce anesthesia and unconsciousness, but in addition it affected the lower centers and in this respect chloroform may be considered the worst of the four anesthetics used.

The changes in the specific gravity of the blood of pigeons during emotional excitement. L. B. NICE and D. FISHMAN (by invitation). Department of Physiology, The Ohio State University.

The specific gravity of the blood of pigeons was determined in the quiet state and during emotional excitement.

The blood was obtained from a puncture of a wing vein of each bird in the quiet state and the specific gravity measured by means of the falling drop method of Barbour and Hamilton. After having determined the specific gravity, the pigeon was then excited by stimulation with a weak Faradic current. Blood was again obtained from a wing vein and the specific gravity measured. About 10 minutes intervened between obtaining the blood in the quiet and excited state in each subject.

It was found that a definite increase in the specific gravity of the blood of each one of our 28 pigeons took place after emotional excitement. In the quiet state the specific gravity averaged 1.0524 and after excitement 1.0548. This gives an average increase of 0.0024 in the specific gravity after excitement.

Effects of local chemical applications to the floor of the fourth ventricle on breathing. HAYDEN C. NICHOLSON and SIDNEY SOBIN (by invitation). Department of Physiology, University of Michigan.

These experiments have been performed upon dogs lightly anesthetized with morphine and urethane. Applying a small piece of filter paper soaked in one or two per cent cocaine solution, or applying a small amount (0.01 to 0.04 cc.) of such a solution to the floor of the fourth ventricle in the region of the calamus scriptorius depresses respiration. This depression is evidenced chiefly by a decrease in amplitude of respiration which is occasionally so marked as to cause apnea. The effects on respiratory rate are less marked. There is ordinarily a slight increase in rate which may be preceded by a transient decrease. There is a sharp drop in blood pressure usually accompanied by a slowing of the heart. The latent period of all these effects is very brief, frequently three seconds or less. Cocaine produces these effects with either intact or blocked vagi. Blocking the vagi has the same effects following the application of cocaine as before its application. Stimulation of the central end of a blocked vagus is apparently equally effective in inhibiting respiration before and after the application of cocaine. Carbon dioxide administered intratracheally seems to be just as effective as a respiratory stimulant when the respiration is depressed by cocaine as normally. However the acceleration of respiration ordinarily produced by stimulation of a sensory nerve such as the

saphenous is much less marked or absent following application of cocaine, though there may be a marked increase in respiratory amplitude.

A small amount of a 1 per cent lactic acid solution applied to the calamus increases the rate and decreases the amplitude of respiration.

Length-tension relationships in contractures of skeletal muscle. DAVID W. NORTHUP (introduced by E. J. Van Liere). Department of Physiology, West Virginia University School of Medicine.

In order to throw additional light on the mechanism of contractures, the relation between the initial length of the muscle and the tension developed of the contracture was investigated. To induce contractures, various agents were used: potassium ions, lactic acid, acetyl choline, and heat.

It was found that when potassium ions are used, the tension of the contracture increases as the length of the muscle is increased, within limits; this is parallel to the behavior of the muscle in normal contraction. This is also true in the case of the lactic acid contracture. Heat contracture is followed by rigor, so that the tensions had to be compared in different muscles by a statistical method. Here also the tension developed increases with the length of the muscle.

In acetyl choline contractures, the tension developed is independent of the length of the muscle, indicating a different mechanism in this type of contracture.

The source of cortical potentials. JAMES O'LEARY (by invitation) and GEO. H. BISHOP. Washington University Medical School, St. Louis.

Considerable attention has been paid recently to localization of cortical potential fields horizontally, but little to localization of potential sources vertically in the layers of the cortex. It seemed possible, since the potentials of the optic cortex, for instance, are practically coincident over the whole striate area, that leads accurately located at different depths might record different components, even though such leads would be close together vertically. Provided electrodes were placed above and below a horizontal layer acting as a unit, it would matter little how slightly above or below the layer these electrodes were placed, since the layer would act as a *charged surface*, each active element preventing the shunting of parallel active elements. Electrodes at different depths might then record different components relatively discretely.

Such is found to be the case. Steel needles insulated except at the tips are thrust tangentially into the striate area of the cortex to different levels, and records obtained from different pairs, both of the spontaneous rhythms and of responses to stimulation of the optic nerve. The positions of the electrodes are checked afterward in sections with reference to the cell layers penetrated. The more prominent repetitive response to stimulation takes place in the most superficial layers, the character of the results being consistent with the notion that the pathway divides and activates the upper and lower layers independently. A marked spontaneous rhythm can be recorded at levels below that at which a repetitive response to stimulation is obtained. This spontaneous response is decreased or inhibited by stimulation, for the duration of the repetitive response to stimulation in the superficial layers.

The stimulation of the end organs of the skin by radiation. T. W. OPPEL and J. D. HARDY (introduced by E. F. Du Bois). New York Hospital and the Department of Medicine, Cornell University Medical College, and the Russell Sage Institute of Pathology, New York.

We have studied the quantity of far infra red, near infra red, and visible light, necessary to produce a sensation of warmth when irradiated to the forehead of white human subjects, and have measured the heating effects of radiation from these sources on skin surface. From these data, calculations of the skin temperature elevation at the time of stimulation have been made.

The spectral distribution of the sources of near infra red, far infra red, and visible light have been determined spectrometrically. The forehead was placed behind a circular aperture of 4 cm. diameter so that only this area was irradiated. The intensity of radiation and skin temperature were measured by radiometers.

The skin temperature changes produced by various quantities of radiation from the various sources have been studied. From these data the temperature change produced by the quantity of energy just sufficient to produce sensation has been calculated. This temperature change was about 0.001°C. per second and could not be measured directly.

The results show that the minimal amount of energy capable of producing sensation is about 0.0086 gm. cal./sec./cm^2. for visible light, 0.0060 gm. cal./sec./cm^2. for near infra red, and 0.0028 gm. cal./sec./cm^2. for far infra red. The surface temperature changes produced by these energies are approximately the same.

Evidence that the cause of fatigue is electrical. O. S. ORTH (by invitation), H. W. NEILD (by invitation), H. M. SCHAMP (by invitation), J. E. SULLIVAN (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana, Illinois.

The gastrocnemius muscle of a frog with its sciatic nerve attached was carefully removed. One non-polarizable electrode was placed on the nerve and another on the muscle. A delicate galvanometer was connected with the electrodes. The nerve was found to be electronegative to the muscle and the galvanometer indicated a flow of current of 1 to 2 micro-amperes from the muscle to the nerve. A stimulating electrode was placed on the nerve near its end and the nerve was stimulated with submaximal induction shocks until the muscle no longer contracted. The effect of the stimulation was to produce progressive decrease in the difference in potential between the nerve and the muscle and when the muscle finally failed to respond to the stimulus the nerve was only slightly electronegative to the muscle.

The stimulating electrode was then removed from the nerve and placed on the muscle and the muscle was stimulated directly with submaximal induction shocks until it no longer responded to the stimuli. The direct stimulation of the muscle caused a still further decrease in the difference in electrical potential between the nerve and muscle and finally a reversal of polarity, the muscle becoming electronegative to the nerve and the galvanometer indicated a flow of current of 1 to 2 micro-amperes from the nerve to the muscle.

The decrease in the difference of potential between the nerve and muscle

during the production of fatigue and finally a reversal in polarity is attributed to an accumulation of negative ions in the muscle probably the negative lactic acid and phosphoric acid ions. As a matter of fact it was found that painting the muscle with a weak solution of either of these acids decreased the positive potential of the muscle and caused a reversal of polarity just as the stimulation did.

In another connection (Mechanism of Pathologic Calcification, Archives of Pathology, Vol. 20, 1935) one of us has presented evidence to show that the electronegativity of injured muscle as well as contracting muscle is due to the negative phosphate ions arising from the hydrolysis of creatine phosphate and adenylpyrophosphate.

Decorticate polypneic panting and sham rage in the cat: their separate central mechanisms in the diencephalon. F. J. OTENASEK and J. L. LILIENTHAL, JR. (introduced by Philip Bard). Department of Physiology, Johns Hopkins School of Medicine.

After acute ablation of cerebral cortices in the cat there ensues, upon removal of the anesthetic (ether), a group of activities which has been termed sham rage (Cannon and Britton, Bard). The emotional display is paroxysmal, and during the quiescent intervals marked polypneic panting occurs spontaneously at normal body and environmental temperatures. This respiratory activity is characterized by to-and-fro movements of the tongue with retraction of the labial commissures synchronous with respiration, rapid rate (100 to 300 per min.) and diminished volume of tidal air.

Once polypneic panting is established at normal body temperature, raising or lowering body temperature produces an appreciable variation in respiratory rate, but such change is not accompanied by any modification of the essential characteristics of the polypneic panting; these remain when the rate is depressed markedly by lowering body temperature three or four degrees Centigrade below the normal range.

Decorticate sham rage is dependent upon the ventro-caudal portion of the diencephalon (Bard, 1928). Our acute experiments show that sham rage and polypneic panting develop after a frontal transection which leaves intact the caudal fourth of the diencephalon. Neither occurs when the transection is caudal to the level of transition between diencephalon and mesencephalon. In such decerebrate animals heating produces an elevation of respiratory rate but does not induce true panting. When the section passes from the anterior edges of the superior colliculi to any point between the chiasm and a level 1 mm. rostral to the mammillary bodies sham rage develops without polypneic panting. Typical decorticate polypneic panting without sham rage has followed sections beginning 3 mm. rostral to the pineal and striking the base through the middle of the mammillary bodies.

It is concluded that the central mechanism responsible for decorticate polypneic panting is separate from and located within the diencephalon dorsal to the mechanism concerned in sham rage.

Uterine motility in the rat as revealed by a cannulation-suspension method. E. E. PAINTER (by invitation) and O. G. HARNE. Department of Physiology, University of Maryland, School of Medicine.

Uterine motility in the rat has been studied by a method employing both

cannulation and suspension of the uterus. This method makes it possible to make simultaneous records of the activity of the longitudinal muscles and the variations in intra-uterine tension resulting from the activity of both the longitudinal and circular muscle systems.

Intra-uterine tension is not altered appreciably when longitudinal contractions are accompanied by relaxation of the circular muscles; but when this coördination is absent, and the contraction phases of both muscle systems are synchronized, relatively high tensions develop, which may reach 25 mm. of mercury.

Intra-uterine tension may rise or fall in the absence of longitudinal contractions, demonstrating that circular muscles have an independent rhythm. This activity of the circular muscles produces periodic changes in intra-uterine tension at the same rate as that observed for longitudinal muscles, suggesting that the pace can be set by the circular muscles.

In lightly anesthetized animals intra-uterine tension changes below 10 mm. of mercury do not call forth body movements; but changes above 15 mm., the result of incoördinate muscle activity or of artificial distension, will bring about evidence of discomfort. The administration of adrenalin or ephedrin in doses smaller than those which affect blood pressure will prevent the generation of high tension.

A simple method for recording splenic volume changes in the intact, unanesthetized dog. LAURENCE L. PALITZ and ROSALYN P. MORSE (introduced by A. B. Luckhardt). Physiological Laboratories, University of Chicago.

Under ether anesthesia and aseptic precautions, a laparotomy is made in the left hypochondrium above the spleen. The spleen is brought out to the surface after ligating and severing the upper part of the gastro-splenic omentum to give freedom of movement. The omentum is drawn out and placed around the spleen, a few stitches holding it in place. Being careful to allow sufficient room for the vessels to lie unobstructed, the three layers of muscle are sewed separately, a space between the external oblique and the skin is made with a finger and the spleen placed into it. The skin is then sewed over the spleen.

Allowing three weeks for recovery, the rubber bladder of a sphygmomanometer cuff is placed over the spleen and held in place by tying with tape or by wrapping the cuff around the abdomen. One outlet of the bladder is attached to a water manometer and through the other a measured amount of air is introduced. Splenic volume changes are determined by noting changes in the level of the record. Rhythmical contractions occurring about one a minute are plainly discernible. A simultaneous respiratory record is taken to rule out changes due possibly to respiration.

To test the apparatus intravenous injections of adrenalin (2 cc.-1:100,000) were administered whereupon the level dropped 27.3 mm. (average of thirteen experiments). The splenic volume returned to normal in 16.2 minutes (average). Saline administered intravenously caused no change. To rule out abdominal changes the bladder of the sphygmomanometer cuff was placed on the opposite side. Adrenalin administered under these conditions caused no change in the level of the record.

Administration of other drugs revealed a definite contraction with pilocarpine. Four cubic centimeters of 1:100,000 solution caused a drop

of 20.1 mm. (average of six experiments), the level returning to normal in one-half to one and a half hours. However, atropine, (1 cc. of 1:1000) injected at the point of greatest contraction returned the spleen to its normal size in seven minutes. Atropine alone dilates, at first, then contracts the spleen slightly. Further work on the action of these and other drugs is contemplated.

The influence of dietary salts on parathyroid tetany in the albino rat. MARY C. PATRAS (by invitation), E. A. GALAPEAUX (by invitation) and R. D. TEMPLETON. Departments of Physiology, University of Chicago and the Departments of Physiology and Medicine, Loyola University School of Medicine.

This report summarizes the results obtained on 242 albino rats showing the relative values of a balanced salt mixture, calcium chloride, and calcium hydroxide on the prevention of parathyroid tetany. The animals used were taken from the University of Chicago and Loyola Medical School colonies (on a diet of Purina Fox Chow ad libitum with meat and bread once a week), weaned at the age of 21 days and thyroparathyroidectomized between the ages of 24 to 30 days inclusive. Immediately following the operation the animals were divided into three groups and placed in a room kept at from 88 to 94°F. The first group received a diet consisting of casein 18 per cent, starch 47 per cent Fleischmann's yeast 15 per cent, unsalted butter 15 per cent, cod liver oil 2 per cent, and a balanced salt mixture (Harris) 3 per cent. The second group received the same diet except for the substitution of 3 per cent calcium hydroxide for the balanced salt mixture. The third group received the same except for the substitution of an equivalent amount of calcium as calcium chloride for the calcium hydroxide.

Five examinations were made of the animals at fixed intervals during the 48 hours following the operation. They were classified as surviving without tetany, surviving with tetany, or dead. Rats were considered as showing tetany if spontaneous stiffening of a leg was seen or if such could be elicited by one-half minute of excitement caused by suspending the animal by its tail.

DIETARY SALTS	NUMBER OF ANIMALS	SURVIVING WITHOUT TETANY		SURVIVING WITH TETANY		DEAD	
		Number	Per cent	Number	Per cent	Number	Per cent
Salt mixture (Harris), 3 per cent	76	11	14.5	20	26.3	45	59.2
$CaCl_2$, 4.5 per cent	83	27	32.5	6	7.2	50	60.0
$Ca(OH)_2$, 3 per cent	83	56	67.4	2	2.4	25	30.1

The results show that calcium hydroxide is superior to calcium chloride or the balanced salt mixture in the treatment of the parathyroprivic condition.

The movements and inhibition of the empty stomach of the sea hare (Tethys californicus). T. L. PATTERSON. Department of Physiology, Wayne University, Detroit.

The sea hare derives its popular name from the prominent character of the front pair of tentacles, which somewhat resemble the ears of a hare. This species of mollusc is a vegetarian, devoid of any shell, and may attain a weight of fifteen pounds or more. This work was conducted at the Hopkins Marine Station of Stanford University where the animals were kept in a large vivarium provided with running sea water. The introduction of the gastric balloon was accomplished by anesthetizing the animal in a solution of two per cent. ether in sea water in a darkened chamber and then passing the balloon and the attached rubber tubes down the esophagus while the animal's muscles were relaxed. Similar to other smaller species of gastropods employed (*Haliotis rufescens* and *Ariolimax californicus*) the gastric contractions in this form were continuous exhibiting an average rate of five to six contractions per minute. Variations in gastric tonus also occurred and the strength of the contractions were dependent on the duration of the period of the fast. Weak acid or alkali when introduced directly into the stomach produced the characteristic type of gastric inhibition.

Effects of strychnine on peripheral nerve. H. B. PEUGNET and G. E. COPPÉE (introduced by Joseph Erlanger). Physiological Department, Washington University School of Medicine.

Method. Electron oscillograph; excised sciatic-digital nerves of *Rana pipiens*, permitting study of single "alpha" axon responses. Strychnine sulphate in bicarbonate-free Ringer.

Results. Strength-duration curves: Rheobase generally lowered by concentrations less than 1:1,000,000; generally raised by stronger solutions. Changes of chronaxie were haphazard and unrelated to concentration.

Cathodal polarization curves usually showed augmentation of depression phase, with stronger solutions.

Action potential: (single "alpha" axons) Concentrations above 1:1,000,000 invariably lowered spike potential (approximately 50 per cent normal at block) and increased total duration (maximum 2–3 times normal). Duration increase is greater than can be explained by slowed conduction, since the potential at the poisoned lead outlasts that of the unpoisoned diphasic element, resulting in an upright "T" wave.

Conduction slowed by 1:100,000; always blocked by 1:500, and frequently by 1:10,000 (small nerves), in 15 to 30 minutes.

Fiber size: "B" wave most susceptible; "alpha" and "C" most resistant.

Absolute refractory period prolonged, roughly paralleling action potential duration. Limit of prolongation difficult to determine because of "conduction-refractoriness" occurring with stronger solutions.

Relative refractory period prolonged, merging with subnormal period.

Subnormal period much prolonged (confirming H. T. Graham). Successive additions of subnormality account for extreme fatigability.

Conduction refractoriness: Second of two impulses starting in normal area fails to pass poisoned area; least interval rises with concentration. This is presumably a result of low potential, high threshold, and slow recovery; a block of "Wedenski" type.

Reversibility decreases with repeated poisoning; poisoning rate rises.

Remarks. It is worthy of note that 1, strychnine concentrations necessary for convulsant action in frogs (Sollman) also have effects upon the threshold and potential of the least susceptible fibers in peripheral nerve;

and 2, strychnine concentrations reported necessary for "curarization" or "motor end-plate block" (about 1:500) readily block nerve fibers in thin trunks, such as those within muscle. This indicates the necessity of caution in the interpretation of results based upon the classical "Claude Bernard technique" for localizing drug action.

Cerebellar symptoms from unsymmetrical lesions after median longitudinal section of the decussations of Forel and of the superior brachium. F. H. PIKE. Department of Physiology, Columbia University. (Read by title.)

The following data are submitted as bearing on the question of cerebellar outflow and the mechanism of compensation for cerebellar lesions.

The midbrain of a cat was incised in approximately the median line from the nucleus of the third cranial nerve (which was involved more on the right side than on the left) to nearly the level of origin of the eighth. At the same time, about two-thirds of the left cerebellar hemisphere was excised.

The immediate effects were severe, and it was nearly a month before the animal could get about at all by itself. At the end of a year, it walked about freely with a slow deliberate gait, climbed up on objects into which it could sink its claws, jumped from a moderate height and broke its fall and cleaned its coat. The great reduction in all motor activity, as compared with a control animal, was apparent. In walking, the effect of the left cerebellar lesion was still apparent in the left fore- and hind-limbs, as compared with the right.

Median section of the decussation of the superior cerebellar peduncle had not completely abolished its functional outflow. Wherever else it may have gone, this outflow could not have exerted its effect through the crossed rubrospinal tract.

The experimental activation of rabbit eggs. GREGORY PINCUS. Biological Laboratories, Harvard University, Cambridge, Mass.

Activation of rabbit ova takes place in two stages. The first stage occurs in the ovary with the first maturation division; this activation occurs normally after copulation and can be induced experimentally 1, by ovulatory or subovulatory dosages of anterior pituitary extracts or prolan; 2, by stimulation of the cervical sympathetics. The second stage normally occurs at fertilization, and is completed by the extrusion of the second polar body. Fertilization can be accomplished *in vitro*, but activation by simple sperm extracts does not occur. The sperm do carry a heat labile substance that effects the dispersal of the follicle cells surrounding freshly shed ova. Activation at this stage can be induced by treating the ova with hypertonic solutions or by exposing the ova to high temperatures (45°C.) for a few minutes. Ova so activated can be transplanted to the fallopian tubes of recipient females, and the subsequent development followed.

The comparison of urea with urea + ammonia clearances in acidotic dogs. ROBERT F. PITTS. Department of Physiology, New York University College of Medicine.

Comparisons have been made in the dog of the clearance of urea nitrogen, urea plus ammonia nitrogen, and creatinine under conditions of marked

acidosis induced by the administration of hydrochloric acid. Dogs were maintained on a low protein diet and comparisons were first made at low levels of plasma urea; urea was then administered and the comparisons were repeated with high plasma urea. Under these conditions the urea + ammonia clearances are much higher, relative to the creatinine, at low plasma levels than at high. The urea clearance alone maintains approximate constancy relative to creatinine at both high and low plasma urea values.

A method of temperature analysis of endocrine function. CHARLES M. POMERAT and M. X. ZARROW (introduced by Hudson Hoagland). Biological Laboratories, Clark University, Worcester.

Measurements of gas exchange were made by the direct method of Warburg using a constant-volume type of respirometer. Special vessels of approximately 30 cc. capacity, including a side-arm chamber for NaOH, were designed to accomodate the newt, *Triturus viridescens.* A graduated pipette affixed directly above the NaOH container by means of ground glass connections permitted the admission of HCl. By means of this device the CO_2 dissolved in the NaOH during the O_2 consumption period could be immediately released and the CO_2 production measured for the same time interval.

O_2 consumption and CO_2 production values were secured for a series of normal animals at temperatures ranging from 6° to 30°C. Temperature characteristics of approximately 16,800 ± calories were obtained for both the O_2 and CO_2 data when plotted according to the Arrhenius equation. This value is of the order of that which has been repeatedly obtained by other workers for cellular metabolism.

This method suggests a new approach to the problem of endocrine control of metabolism. Temperature analyses following the above technique using hypophysectomized, thyroidectomized, and castrated animals as well as animals having received various types of replacement therapy are now in progress. The purpose of these investigations is to determine shifts in the value of the temperature characteristics which may occur as a function of change in the endocrine balance.

Electrical timing and recording devices. F. H. PRATT and W. T. PORTER. Boston University and Dover, Massachusetts. (Demonstration.)

A signal magnet of compact form, providing rapid and precise adjustment of the vibrator for tension and for range of movement.

Magnetic repulsion drive and non-sparking mercury contact for a vibrating-reed variable interrupter.

An improved form of armature as applied to the pendulum of the electrically driven clock.

The relation of pancreatic juice to the fatty infiltration and degeneration of the livers of depancreatized dogs. JOHN VAN PROHASKA (by invitation), HERMAN P. HARMS (by invitation) and LESTER R. DRAGSTEDT. Department of Surgery of The University of Chicago.

The observations of Fisher (1934) and of Allan, Bowie, Macleod, and Robinson (1924) and others that completely depancreatized dogs adequately treated with insulin usually die within two months with extensive

fatty infiltration and degeneration of the liver was confirmed. The absence of pancreatic juice from the intestine is apparently not the cause of this liver damage since it was not observed in animals with complete pancreatic fistulae or in dogs whose pancreatic ducts had been ligated and the pancreas extensively degenerated. Two hypophysectomized depancreatized dogs, although they ate less of the standard diet, developed the fatty changes sooner and more extensively than the depancreatized animals. The administration of fresh activated dog pancreatic juice in amounts from 100 to 1100 cc. per day did not prolong the lives of depancreatized dogs nor prevent the development of the typical liver changes. The oral administration of as little as 25 grams of fresh beef pancreas per day was found sufficient to either prevent or to cure the fatty infiltration and to permit survival. Ten grams of fresh pancreas was not sufficient. The oral administration of 200 mgm. of choline choloride per day did not prevent the fatty changes in the liver or permit survival. The daily administration of 1000 grams of beef pancreas that had been boiled for 15 minutes did not prevent the development of typical fatty changes in the liver.

The effect of weaning upon the excretion of calcium in the urine of lactating rats. L. I. PUGSLEY (introduced by J. B. Collip). McGill University, Montreal, Canada.

The weaning of lactating rats caused an increased excretion of calcium in the urine which was maintained for four days. It then returned to the normal level. When the mammary glands of lactating rats were removed this increased excretion of calcium did not occur. It was found that the calcuria was dependent upon the time of lactation. Rats weaned at the day of birth of the litter showed a very small increased excretion of calcium while those in which the litter was allowed to suckle for ten days before weaning showed an increased excretion of calcium in the urine of sixty times the control value.

Calcium and iodine balance as related to thyroid function. ITALO D. PUPPEL (by invitation) and GEORGE M. CURTIS. Department of Medical and Surgical Research, The Ohio State University, Columbus, Ohio.

The calcium and iodine balance was determined on two normal individuals, on one patient subsequent to a total thyroidectomy and on two patients with hyperthyroidism. A constant diet was selected and begun by each individual two or three days prior to commencement of investigation. It was of a low-calcium, sufficient iodine content, adequate in other respects and as attractive as possible. One patient with hyperthyroidism was maintained on a high calcium intake. Other qualities of the diet will be reviewed.

The serum calcium and phosphorus of all five individuals varied within normal limits. In the patient with hypothyroidism there was an increased retention of calcium. In the two normal individuals on a low calcium diet the calcium balance was negative. In the two patients with hyperthyroidism there was an increase in the excretion of calcium. In one the increase was almost entirely in the feces. In the other the increase was both in the urine and feces. This resulted in a continuous negative calcium balance greater than normal.

As determined by Cole the blood iodine of the two normal individuals averaged 11 micrograms per cent. In the patient with hypothyroidism the blood iodine was decreased to an average of 5 micrograms per cent. The blood iodine of the two patients with hyperthyroidism averaged 24 micrograms per cent, which is greatly increased. The normal individuals were in a continuous positive iodine balance. The hypothyroid patient showed a negative balance The urinary loss of iodine was greater than the fecal excretion. In one patient with hyperthyroidism the iodine balance was continuously negative. In the other patient it was also less than that of the two normal controls. In both the increased fecal excretion of iodine was greater than the increased urinary loss, thus differing from the normal and hypothyroid individuals.

In the hypothyroid patient the decrease in circulating iodine was accompanied by a decrease in calcium excretion. In the two patients with hyperthyroidism the increase in calcium excretion accompanied an increase in circulating iodine.

The physiological importance of the gastric body and fundus. J. P. QUIGLEY, I. H. EINSEL (by invitation), F. R. BING (by invitation) and R. F. HANZAL (by invitation). Western Reserve University, Medical School.

The body and fundus of the stomach are significant chiefly as a reservoir, as a source of a digestive secretion and, according to Klein, as the site of a "gastric pace maker." Employing animals from which the gastric body and fundus were extirpated, we have investigated the importance of these structures and the functions they subserve.

Dogs so prepared form a gastric secretion following histamine administration which contains no free hydrochloric acid (100 cc. required 10 cc. N/10 HCl to react acid to Topfer's reagent). Pepsin is present only in traces. Thoroughly cooked food if eaten slowly is well digested and absorbed and normal weight and excellent condition is maintained. The digestion and absorption of raw or incompletely cooked food or rapidly eaten food is less satisfactory, particularly in respect to fats.

The gastric remnant exhibits continuous motility (balloon method) during fasting periods (rest periods were absent during extended intervals of observation). The influence of the body and fundus thus appears to be more inhibitory than augmentory. Tonus of the remnant is low but this is probably dependent on a sectioning of the gastric vagus fibers incident to the operation.

The blood serum iron curve following intragastric administration of ferrous chloride to normal and operated dogs demonstrates practically normal iron absorption in the latter animals. The absorption of iron in either case is only slightly augmented by the simultaneous administration of hydrochloric acid. The operated dogs show a low hemoglobin (10 gm. per 100 cc. of blood) and erythrocyte count (6–6.5 million per cu. mm.).

The importance of gastric juice in the absorption of calcium by dog and man. J. P. QUIGLEY, I. H. EINSEL (by invitation) and J. W. MULL (by invitation). Western Reserve University, Medical School. (Read by title.)

Absorption of calcium from the gut is apparently limited to calcium in ionic form and is therefore dependent on its existence in inorganic forms, the extent of its recombination into insoluble forms (e.g., soaps and phos-

phates) and the reaction of the intestinal contents. Gastric juice and especially hydrochloric acid should therefore play an important rôle in calcium absorption.

We investigated this problem in dogs whose secretion of hydrochloric acid and pepsin was entirely prevented by extirpation of the gastric body and fundus. The blood calcium curves following the intragastric administration of calcium gluconate or calcium lactate to operated animals was compared with those obtained under similar conditions from normal animals. The results were sufficiently similar to demonstrate the absorption of calcium by animals with experimental achylia gastrica at a rate closely approximating normal. Since the simultaneous administration of hydrochloric acid and calcium salt to either group elevated the blood calcium curve only slightly, the conclusion appears justified that under these experimental conditions hydrochloric acid was not essential for calcium absorption.

Blood calcium curves obtained in clinical cases of achlorhydria or achylia gastrica after the oral administration of 10 grams of calcium gluconate or calcium lactate indicate a definite but limited absorption of calcium in such subjects.

Changes in the vitamin A and carotene content of the livers of dogs following depancreatization or ligation of the pancreatic ducts. ELAINE P. RALLI and ARTHUR C. PARIENTE (by invitation). The Nutrition Laboratories of the Department of Medicine, New York University College of Medicine.

The effect of ligation of the pancreatic ducts or of depancreatization on the carotene and vitamin A content of the liver was studied in a group of 6 dogs. The animals were placed on a basic diet to which was added either carotene with mazola or vitamin A as cod liver oil. Two dogs received no source of the vitamin but an equal amount of fat as crisco. After a period on the diet with one of the above additions, a piece of liver was removed and the animal depancreatized or the ducts ligated. Following operation the animals were maintained on the same diet and after a period of several months another piece of liver was removed. In addition four dogs were observed as normals. These animals received the same basic diets, with the additions of cod liver oil or carotene with mazola, and at operation only a piece of liver was removed. After a period of several months on the diet, another piece of liver was removed. Lecithin (10 grams daily) was given to one normal dog on carotene and one on cod liver oil and to the ligated and depancreatized dogs.

In all animals the liver was analyzed for carotene and vitamin A before operation and at the end of the experimental period. Vitamin A and carotene were extracted by the alkali digestion method. In the earlier experiments the extracted vitamin was estimated by the antimony trichloride test and read in a Lovibond tintometer. Carotene was read directly in the tintometer. In the more recent experiments vitamin A was estimated in a Hilger vitameter and carotene in a Pulfrich photometer. The vitamin is reported in international units and carotene in milligrams per 100 grams of liver.

The livers of the normal dogs had a greater increase in vitamin A following cod liver oil administration than did the livers of the depancreatized

or duct ligated dogs. Lecithin administration did not affect the liver vitamin A in any of the dogs.

Focal stimulation of the interior of the brain with the Horsley-Clarke apparatus. S. W. RANSON, H. W. MAGOUN (by invitation) and K. HARE (by invitation). Neurological Institute, Northwestern University Medical School, Chicago, Illinois. (Demonstration.)

Illustrations of specific responses to localized stimulation of points within the interior of the brain with the aid of the Horsley-Clarke instrument.

Investigations on waltzing and shaking rodents of particular genetic types. A. E. RAUH and H. D. KING (introduced by D. McK. Rioch). Department of Anatomy of Harvard Medical School. (Read by title.)

Waltzing mice, guinea pigs and rats of particular genetic types, and shaker$_2$ (Zavadskaia) mice have been used. All these animals were more active than normals. In addition they showed characteristic, spasmodic attacks, increased by excitement. The waltzing animals whirled rapidly or ran in small circles. Most of the animals whirled or circled both clockwise and counter-clockwise, while in some the direction was fixed. The shakers threw their heads back, jumped, turned a back-flip in the air and landed on their feet. Between attacks no asymmetry in posture was noted.

All animals landed on their feet when dropped. However, all failed to show normal nystagmus when rotated with constant acceleration. The rats, in contradistinction to the mice, showed rotation and verticle deviation of the eyes in response to appropriate positions of the head in space.

The mice and guinea pigs failed to respond to sound, while the rats responded normally.

Placing reactions of the guinea pigs and rats were performed more quickly than the normal animal executed them, and their hopping reactions were slightly more accurate.

Postural changes on an inclined surface were absent in the guinea pigs and present in the rats.

In the brains of three mice examined, large capillary sinuses have been found, particularly throughout the vestibular nuclei. No other gross abnormalities were found in these brains.

The rôle of the thyroid in the calorigenic action of vitamin D. C. I. REED, HARRY DEUTSCH (by invitation) and H. C. STRUCK (by invitation). University of Illinois, College of Medicine, Chicago.

It has been shown that vitamin D will increase the metabolic rate of normal dogs and rats and that the oxygen consumption of isolated frog muscle is augmented. It has also been shown that parathyroid extract will not increase the metabolic rate of normal dogs. In an effort to explain this action, two dogs were trained for metabolic rate determination, then subjected to complete thyroparathyroidectomy and studied for six months. The administration of vitamin D in amounts as high as 100,000 U. S. P. units per kilogram of body weight per day did not effect any significant alteration in the metabolic rate. These results suggest that the calorigenic effect of the vitamin involves the thyroid.

The action of strychnine on anuran lymph hearts. MARION A. REID (introduced by F. H. Pratt). Department of Physiology, Boston University School of Medicine.

Schiff (1885) reported that a posterior lymph heart, mechanically freed by denervation of the immediate muscular area, became tetanized under strychnine along with the muscles of the intact side. This is not confirmed: the organ has always remained in diastole throughout a convulsion; the interruption usually outlasting it, but giving place to the normal rhythm before a succeeding spasm. The same results occurred in intact animals (alcohol, nembutal or ether), and under wide variation of the convulsive dose (0.75–5.0 mgm. per kgm.).

Since inhibitory impulses to the lymph hearts proceed from the medulla, the vagus being the afferent pathway, this source of inhibition was investigated. During heavy etherization, or immediately after pithing brain and medulla, inhibition was frequently shortened. However, when the spinal cord was hemisected at the caudal border of the medulla there was no consistent difference in duration of the inhibitory pause of contralateral organs. Vagotomy on one side was also ineffective. Cord transection at the border of the medulla, with 1 to 3 days for recovery, produced no change in duration of inhibition. In view of the importance of local afferent nerve impulses in somatic muscular responses to strychnine, the anterior lymph-heart area was de-afferented in one preparation by cutting the dorsal roots of the 3rd and 4th spinal nerves. In this, a spinal animal, convulsions characteristically interrupted the beat.

Synchronism of homolateral organs (Pratt and Reid, 1932) was unchanged by strychninization. No acceleration appeared except in moribund animals with irregular and sluggish beat. Once the posterior beating continued throughout the brief reactions induced by touching the skin (as with sub-convulsive doses) but was inhibited during convulsions induced by jarring.

Since in normal lightly anesthetized frogs general body movements, spontaneous or stimulated, do not affect the rhythm of the lymph hearts, it is suggested that strychnine somewhere facilitates the activity of inhibitory impulses normally released through the afferent activity of the vagus. If strychnine acts on pre-motor elements it may be assumed that inhibitory intraspinal pathways may be stimulated during that discharge which simultaneously tetanizes skeletal muscle.

Physical and hormonal factors in uterine growth. SAMUEL R. M. REYNOLDS and SANFORD KAMINESTER (by invitation). Long Island College of Medicine.

The effect of graded degrees of chronic uterine distention on uterine structure is studied during the second and third week after ovariectomy in mature virgin rabbits. In some instances, no replacement with ovarian hormones is made while in others the effect of oestrone and progesterone is examined. Adequate controls of acutely distended uteri are made for comparison with the sites of chronic distention. It is found that hyperplasia of the muscle, an increase in connective tissue and growth of the endometrium take place in the absence of all ovarian hormonal influence but resulting from physical distention alone. Undistended portions of the same uteri show characteristic changes of castration atrophy. Slides will

be shown and a comparison made of relative differences between chronically distended uteri with and without the ovarian hormones present. The degree of uterine growth will also be correlated with the degree of uterine distention.

Experimental efforts to learn whether inulin is secreted by the tubule of the mammalian kidney. A. N. RICHARDS, B. B. WESTFALL (by invitation) and PHYLLIS A. BOTT (by invitation). Laboratory of Pharmacology, University of Pennsylvania.

In dogs and rabbits, by a variety of procedures, the renal blood pressure has been lowered to a level believed to be too low to permit glomerular filtration. Inulin, in combination with other substances, some of which (e.g., phenol red) are believed to be secreted by the tubule, was then introduced into the renal circulation. After varying periods of time the renal vessels were then perfused at high pressure with blood or Locke's solution, not containing these substances, in the hope that the urine first obtained from the ureter would show by its composition that one or more of the added substances had found access to the lumina of the tubules during the low blood pressure period. The results were such as to strengthen belief that phenol red under these conditions is secreted and inulin is not.

Increased calcium appetite of parathyroidectomized rats. CURT P. RICHTER and JOHN FULTON ECKERT (by invitation). Psychobiological Laboratory, Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md.

It is a well known fact that parathyroidectomized animals have a marked calcium deficiency which can be corrected by calcium feeding. It is of interest to know whether they voluntarily take enough calcium to keep themselves free from symptoms of parathyroid deficiency; in other words, whether, after parathyroidectomy appetite for calcium is dependent on the calcium need just as after adrenalectomy salt appetite has been shown to be dependent on the salt need.

In a preliminary series of experiments, six rats on a calcium free diet were given a free choice of drinking tap water or a 2.4 per cent calcium lactate solution. The intake of calcium lactate increased from an average of 0.1 gram per day for the ten day period immediately preceding parathyroidectomy to a daily average of 0.4 gram thirty days later. Essentially the same results were obtained in sixteen additional animals. It is thus demonstrated that parathyroidectomized animals do show a marked increase in calcium appetite.

On the identity of the hormone causing ovulation in the rabbit. OSCAR RIDDLE, ROBERT W. BATES (by invitation), ERNEST L. LAHR (by invitation) and CLARENCE S. MORAN (by invitation). Carnegie Institution, Station for Experimental Evolution.

Minimal ovulation dose (M. O. D.) was determined for several more or less purified preparations capable of inducing ovulation in the normal $3\frac{1}{2}$ to $4\frac{1}{2}$ month non-estrus New Zealand rabbit (130 tests). These same preparations were injected into immature male ring doves and their content of certain pituitary hormones determined (44 tests); in this report only the quantitative growth response of the testis requires consideration, and

this response is evidently induced by the follicle-stimulating hormone (F. S. H.) without augmentation by luteinizing substance.

The following preparations yielded an increase in percentage weight of testis in the dove essentially in direct proportion to their M. O. D. in the rabbit: mare's serum, our own F. S. H.+, the F. S. H. and the L. H. of Fevold and Hisaw, Schering's thyreotropic, Antuitrin and Phyone. But pregnancy urine and placental preparations have much greater capacity to induce rabbit ovulation than to induce testis growth, and others have shown that such preparations induce release of F. S. H. from the pituitary of the rat. Since the rabbit's pituitary is apparently in similar condition to release F. S. H.—though that of the immature dove is not (Riddle and Schooley)—this result can be in agreement with the results obtained from the above-mentioned true pituitary derivatives in indicating that F. S. H. induces ovulation in the rabbit. Neither presence nor apparent absence of prolactin or thyreotropic modified the relationship between M. O. D. and percentage increase in dove testis size.

This action of F. S. H., in this type of rabbit, is uninfluenced by pituitary L. H. (of Fevold and Hisaw) except for the additive effect of the F. S. H. content of the latter. Heat slowly destroys, tryptic digestion rapidly destroys, the capacity of any preparation to induce both rabbit ovulation and growth in the dove testis. These data exclude any need for the postulation of a unique or distinct "ovulation hormone" in the rabbit's pituitary and make it highly probable that F. S. H. induces ovulation in this particular rodent.

An attempt to stimulate the thyroid gland in rats by exposure to cold. G. C. Ring. Department of Physiology, Harvard Medical School, Boston.

Benedict and Macleod have found that "rats studied at 28°C. after living 24 hours at this temperature have a lower heat production than rats studied at 28°C. after living 24 hours at 21°." Since histological evidence indicates that the thyroid is stimulated by cold, it seemed possible that the increase in metabolism might be caused by this gland.

In order to obtain marked differences in basal rate as a result of exposure to different environmental temperatures, the following procedure has been adopted. Rats were kept at 35°C. for one week and the heat production at 28°C., measured on the first and fourth days thereafter. The next week the animals were kept in a refrigerator at 6 to 8°C. A second series of basal rates measured at 28°C. showed a greatly elevated metabolism which did not return to normal for several days.

Animals without thyroid glands showed the usual reduced heat production. When subjected to the procedure outlined above, cold was found to stimulate their metabolism to the same extent as that of control animals.

Concerning the distribution of the Purkinje substance and certain physiological implications dependent thereon. Jane Sands Robb. Department of Pharmacology, Syracuse University College of Medicine, Syracuse, N. Y.

The sheath surrounding the Purkinje substance in the beef heart has been injected with India ink. Subsequently the specimens have been dissected (or cleared), to show the ramifications of the system. The

observation of Cardwell and Abramson (Am. J. Anat. **49**: 167, 1931) that in the right ventricle the myocardial layers of the Purkinje substance are parallel to the subendocardial network has been confirmed. The additional observation is made that these main Purkinje pathways are parallel to the muscular fibers. In the left ventricle also, the main Purkinje pathways are parallel to the direction of the muscle fibers.

If one accepts the belief that conduction is by way of Purkinje tissue, then one may also believe that conduction is parallel to fiber direction of the muscle. Such beliefs are incompatible with the theory of radial penetration of the action current.

The distribution of the Purkinje substance. JANE SANDS ROBB. Department of Pharmacology, Syracuse University College of Medicine, Syracuse, N. Y. (Demonstration.)

Dissections and cleared specimens, following injection of the sheaths with India ink, have been prepared. These pathways are seen to be parallel to the fiber direction of the muscles of the ventricular walls.

The distribution of carbonic anhydrase in some invertebrates. KATHLEEN ROBERTSON (by invitation) and J. K. W. FERGUSON. Department of Biology, University of Toronto, and Department of Physiology, University of Western Ontario.

The manometric technique of Meldrum and Roughton was used to assay the amount of carbonic anhydrase in the tissues of several marine invertebrates available at the Marine Biological Laboratory, Woods Hole. The test extract was made by grinding the tissues (or in some cases the whole animal) with sand and distilled water, then centrifuging.

The criteria for carbonic anhydrase activity were that the supernatant: *a*, catalyse the evolution of carbon dioxide from the phosphate-bicarbonate mixture; *b*, be inactivated by heating; *c*, exhibit its activity in the water-alcohol fraction after treatment with alcohol and chloroform.

The names of the invertebrates examined appear in the list below, each followed in brackets by the common name (if any), and the name of the respiratory pigment of its blood; Loligo pealei (squid, hemocyanin), Homarus americanus (lobster, hemocyanin), Limulus polyphemus (King crab, hemocyanin), Busycon canaliculatum (snail, hemocyanin), Nephthys (hemerythrin), Arca (erythrocruorin).

The results and conclusions may be summarized as follows: 1. Carbonic anhydrase is present in the tissues or blood of all of these invertebrates regardless of whether they have as their blood respiratory pigment, hemocyanin, hemerythrin, or erythrocruorin.

2. The blood of the squid and lobster contains no carbonic anhydrase, but various tissues, particularly the gills, do.

3. In the case of the blood of limulus, carbonic anhydrase is limited to white cells.

4. The enzyme is not associated with the blood respiratory pigment in these animals containing hemocyanin.

5. It would appear that carbonic anhydrase is always a cellular constituent, as it has never yet been found to occur in fluids free from cells or lysed cells.

Relation of the adrenal glands to experimental pancreatic diabetes. J. M. ROGOFF and H. WARD FERRILL (by invitation). Physiological Laboratory, University of Chicago.

We have repeated and extended the experimental work upon which was based preliminary reports to the effect that unilateral adrenalectomy or interference with epinephrine secretion (adrenal denervation, etc.) ameliorates diabetes in depancreatized dogs. Studies on a more adequate series of controls and quantitative observations on epinephrine output from the adrenals (by the method employed by Stewart and Rogoff) indicate that we were misled in the interpretations of the experimental work, previously reported.

It is now evident, from satisfactory experiments, that partial adrenalectomy or operations for suppressing epinephrine secretion (adrenal denervation, with or without mechanical destruction of medulla), do not prevent or diminish the experimental diabetes produced by total pancreatectomy, in dogs. This conclusion is supported by the following observations: *a*, insulin requirement to control glycosuria is the same for control depancreatized dogs as for those subjected to adrenal operations in addition to pancreatectomy (diet and other conditions being constant): *b*, insulin requirement may be of the ordinary magnitude or may be relatively low in control depancreatized dogs, as well as depancreatized dogs with adrenal operations, regardless of whether the epinephrine output is in the normal range (as determined by Stewart and Rogoff), reduced or suppressed: *c*, insulin requirement may vary within a relatively wide range in one and the same experimental animal at different periods and it may differ in different animals, under similar conditions.

In view of clinical reports on alleged benefit in diabetes from x-ray or denervation of the adrenals, we can report the interesting observation that depancreatized dogs, not subjected to interference with the adrenals but kept on a constant diet and sufficient insulin to maintain glycosuria at a very low level, develop reduced or suppressed epinephrine secretion. Whether this occurs in animals not treated with insulin, and other factors that may affect the adrenals in depancreatized animals, is under investigation.

The electrograms of the pilomotor muscles. A. ROSENBLUETH, H. DAVIS and B. REMPEL (by invitation). Department of Physiology, Harvard Medical School.

The electrograms of the pilomotor muscles of the tail of the cat were found to vary with the position and distance of the recording leads (silver-silver chloride needles) as follows. During the first spike component (I) of the electrogram the lead inserted cephalad on the tail becomes electropositive with respect to the lead inserted caudad. This occurs whether the cephalad lead is the grid or the ground. The same polarity of I is observed when the skin is removed at either or both the points of insertion of the leads.

When either of the leads is inserted at a given point in the tail and the other is placed in successive positions describing a semicircle around it, beginning cephalad and ending caudad, the electric responses first decrease in magnitude until they disappear when the two needles occupy a plane perpendicular to the longitudinal axis of the tail. They then reappear and

increase, but reversed in polarity, so that at the end a mirror image of the first response is obtained.

The magnitude of the electrograms is proportional within limits (10 to 15 cm.) to the distance between the needles along the longitudinal axis.

It is concluded that the electrograms denote an oriented asymmetric depolarization of the muscle cells between the electrodes and that they are additive in series.

The rate of conduction of the nerves may be calculated by measuring the differences in the latency of I at different levels in the tail. Such a calculation yields speeds of about 2 to 4 m./sec. After the time for conduction is subtracted from the latency of I an interval of at least 60 m/sec. remains. This interval is reasonably explained by assuming that I does not precede the chemical mediator liberated by the nerve impulses, but is produced by it.

The rôle of the thymus and pineal glands in growth and development. L. G. ROWNTREE, J. H. CLARK (by invitation), ARTHUR STEINBERG (by invitation), N. H. EINHORN (by invitation) and A. M. HANSON (by invitation). Philadelphia Institute for Medical Research, Philadelphia, Pa., and Faribault, Minn.

Studies on the thymus gland have been carried on over a period of three years and on the pineal gland for approximately two years. The thymus studies have included the following procedures with the following results:

1. The administration of thymus extract (Hanson) to successive generations of parent rats through twelve generations has resulted in accruing acceleration in the rate of growth and development in successive generations of young.

2. Thymectomy in five successive generations of parents has resulted in marked retardation in the rate of growth and mild retardation in the rate of development of the young.

3. Replacement thymus therapy, i.e., the injection of thymus extract through four generations has completely overcome the retarding effect of thymectomy in four generations of parents. Such replacement therapy pushed vigorously leads to acceleration in the rate of growth and development of the young.

4. Frequently repeated homologous fresh thymus implants in three successive generations of thymectomized parents has overcome entirely the retardation in growth and development incident to thymectomy. More frequent implants lead to acceleration of growth and development of the young.

5. Frequently repeated homologous fresh thymus implants in three successive generations of normal parent rats has resulted in marked acceleration in the rate of growth and development of the offspring.

The pineal studies have consisted of the following procedures, with the following results:

1. Pineal extract (Hanson) in six successive generations of parent rats has resulted in accruing retardation in the rate of growth and accruing acceleration in the rate of development of the young—precocious "dwarfism."

2. The effects of pinealectomy in four successive generations of parent rats have been inconstant to date.

As a result of these studies, it appears evident that the function of the thymus gland of the parent rat is concerned with the rate of growth and development of the offspring. The studies on the pineal gland have not progressed to date to the point which permits any statement concerning the function of this gland.

Studies on the thymus and pineal glands. L. G. ROWNTREE, J. H. CLARK (by invitation), A. STEINBERG (by invitation), N. H. EINHORN (by invitation) and A. M. HANSON (by invitation). Philadelphia Institute for Medical Research, Philadelphia, Pa., and Faribault, Minn. (Motion picture demonstration.)

Some recent evidence for a humoral mechanism for peripheral sensory inhibition (adaptation). MORTON A. RUBIN (by invitation) and HUDSON HOAGLAND. The Biological Laboratories, Clark University, Worcester.

It has been proposed by one of us (Hoagland) on the basis of a variety of evidence that cutaneous tactile receptors in frogs' skin become adapted as a result of the accumulation around the sensory nerve endings of K released from epithelial cells by the mechanical stimulus.

Further studies to test this hypothesis have yielded the following results. Histochemically it was found that the potassium in frogs' skin (132 ± mgm. per cent, 128 ± mgm. per cent of which is diffusible) is concentrated in the epithelial cells. A small amount is also present in the gland cells. Very little K, if any, was found in the corium. When the skin was intermittently stimulated by means of an air-jet and the underside of the skin was washed with Ringer's solution, of the ions K, Ca, Cl, and PO_4, only K was found to diffuse out of the skin, as was shown by the presence of an additional amount of K (mean of 0.67 mgm. per cent) in the wash Ringer's solution. The Ringer's solution used to wash unstimulated skin was found to contain no additional K. Bathing the underside of the skin in a solution of Ringer's containing 15 times the normal K content immediately depressed the excitability of the nerve endings as was shown by the subsequent hastened adaptation recorded in the usual way as single axon spikes set up by the interrupted air-jet applied to the skin. Washing the underside of the skin containing the receptor with normal Ringer's solution removed the inexcitability of the single ending produced by the K. Increasing the Ca content of the Ringer's solution 15 times also produced a depression of responsiveness roughly of the same order of magnitude as that of the K depression. This depression, however, did not occur so rapidly, and the application of normal Ringer's did not produce recovery as in the case of the K depression. The otherwise irreversible Ca depression can, however, be removed by bathing the skin in K-rich Ringer's.

The results here described, in addition to the earlier evidence, strongly support the hypothesis that cutaneous sensory adaptation is normally produced by the accumulation of K around the sensory nerve endings, which thus lowers their excitability by reducing the ratio of K inside the branching axon fibers to that outside.

Human ovulation. Exploratory studies. BORIS B. RUBENSTEIN. Brush Foundation. Western Reserve University, Cleveland, Ohio. (Read by title.)

The object of this inquiry is to study ovulation in women, its mechanism, the alterations induced by it, and its temporal associations with other physical and with mental manifestations.

Any test for the occurrence of ovulation must be simple enough to be widely applicable, since 1, the onset of ovulation is an important date in adolescence; 2, it is not yet known how frequently ovulation occurs in humans; 3, certain investigators have asserted, others denied the existence of a fertile and sterile period in each menstrual cycle upon evidence of a definite time relation of ovulation to the subsequent menstruation.

A systematic investigation on four women correlates pulse rate, its response to exercise and drugs, blood pressure, ocular accommodation, gastrointestinal activity, sweating, and voluntary muscle control with data obtained by Papanicolaou's vaginal smear technique. The underlying hypothesis is that ovulation is controlled by a neurohumeral mechanism involving the autonomic nervous system which is so altered by the accompaniments of ovulation that modifications will be expressed in the several organ systems.

Four medical students were selected for regularity of menstrual function and freedom from pelvic pathology. Rectal temperature was taken daily on arising; vaginal smears made; weight taken after voiding. After a light breakfast, the subjects came to the laboratory where the blood pressure, pulse and respiration were measured after fifteen minutes recumbency and after definite exercise,—raising a ten pound weight two feet, five times and at one minute intervals until recovery. A food and activity diary was kept. Daily tests of arm steadiness and of tapping speed were administered. Weekly fluoroscopies of the stomach were made; weekly infra-red photographs of the breasts and measurements of areolar diameter were taken.

Records kept for two complete menstrual cycles indicate a premenstrual peak and midmenstrual minimum of basal temperature. The other studies except the fluoroscopies all yield suggestive data, but so far no definite indication of a relation to ovulation. The gastrointestinal studies have shown that the gastric pattern remains characteristic for the individual, finer changes are not measurable by simple fluoroscopic examination.

Somatic sensory function of the cerebral cortex in the monkey and chimpanzee. T. C. Ruch and J. F. Fulton. Laboratory of Physiology, Yale University, New Haven.

Monkeys and a chimpanzee trained in various somato-sensory discriminations have been tested after single and combined lesions in the parietal and frontal lobes. In the monkey, single lesions of the parietal association area (Brodmann area 7), the postcentral gyrus (areas 3, 1, 2, and 5), and the motor area (incomplete anteriorly) fail to reduce the accuracy of weight discrimination. There is, therefore, apparently no focal representation of this function within the cortical region explored. Destruction of the anterior wall of the central sulcus added to an ablation of the postcentral gyrus, which includes all the "focal zone" of the somatic sensory projection area (Poliak), produced no deficit in weight discrimination. However, a lesion which includes the posterior parietal lobe in addition to the postcentral gyrus induced a gross initial loss of sensory ability which lessened but persisted with extensive retraining. A bilateral parietal

lobectomy and destruction of the anterior wall of the central sulcus reduced but did not abolish the ability to discriminate inclined planes in total darkness. These experiments suggest that somatic sensory functions are widely rather than focally represented in the cerebral cortex of the monkey and that the thalamus suffices for gross discrimination.

The effect of unilateral ablation of the postcentral gyrus upon the ability of the chimpanzee to discriminate degrees of "roughness" (emery paper) has been studied. Initially the accuracy of discrimination was greatly decreased, but careful retraining demonstrated a considerable residual ability. The remarkable fineness of discrimination exhibited preoperatively was completely regained. In the chimpanzee the postcentral gyrus is an important sensory area but apparently forms only a part of the cortical representation of discriminative sensibility in the tactual sphere. These observations point to a progressively greater degree of "encephalization" and "concentration of representation" of somatic sensory function in the primate series.

Carbohydrate and phosphorus changes in prolonged muscular contraction. JACOB SACKS, WILMA C. SACKS and J. R. SHAW (introduced by C. W. Edmunds). Laboratory of Pharmacology, University of Michigan Medical School.

The gastrocnemius muscles of cats under pentobarbital anesthesia were stimulated indirectly *in situ* to give isometric twitches at rates of 1 and 2 per second for periods of 1, 2, 5 and 10 minutes. Changes in glycogen, lactic acid, hexosephosphate, phosphocreatine and adenosine triphosphate were studied.

In muscles stimulated at the rate of 1 per second, relatively little lactic acid was formed. The highest rate of formation was found during the first minute. Less was formed during the second minute; the rate of formation then dropped, to a practically constant one. The maximum concentration in the muscles was reached at 5 minutes. No formation of hexosephosphate or hydrolysis of adenosine triphosphate was observed under these conditions.

In muscles stimulated at the rate of 2 per second, lactic acid formation during the first minute was 3 or 4 times as great as in the other series. During the second minute, the rate of formation decreased markedly, but was still greater than with the slower rate of stimulation. Thereafter, lactic acid formation continued at a rate about twice that found in the other series. The concentration in the muscle remained practically constant after the second minute. A large amount of hexosephosphate was formed during the first minute; after this time the amount present decreased rapidly. Hydrolysis of adenosine triphosphate was apparent in 1 minute and became greater in amount as the contractions continued.

Lactic acid was lost from the muscle throughout the entire period in both series.

Two kinds of "steady state" have thus been found. At a low level of activity, the muscle contains relatively little lactic acid, and no hydrolysis of adenosine triphosphate is found; at the higher level, there is a very high lactic acid concentration together with progressive hydrolysis of adenosine triphosphate. There appears to be a continuous lactic acid production during the steady state, roughly proportional to the degree of activity of the muscle.

Study of depth temperatures in artificial fever and cooled air chambers with especial reference to cooling effect of circulating blood. JOHN J. SAMPSON. Mount Zion Hospital, and Department of Medicine, University of California, San Francisco, California.

Wright and Johnson and other investigators have shown a marked discrepancy in the temperatures of different tissues and particularly a delay in the rise of venous blood temperature in fever artificially produced by typhoid vaccine injections in man.

Using the method of restricting heat loss by blankets and rubber sheets, Epstein and Cohen successfully produced artificial fever for the treatment of central nervous system syphilis. Intravenous, intramuscular, subcutaneous, and skin temperatures were measured by the use of a needle thermocouple on individuals undergoing this artificial fever treatment. The temperatures rose rather uniformly with intramuscular exceeding rectal, which in turn was higher than oral and subcutaneous, with intravenous being lower than all but skin temperature and sometimes falling below the covered surface temperatures. On sponging the patient and rapidly cooling, the fall of intravenous temperature was slower than all others.

Attempting to show the degree and rapidity of the cooling influence of the blood as influenced by exposure to cool air in the lungs and that early temperature fall recorded in cases of pneumonia patients placed in iced oxygen tents were probably due to this mechanism, we recorded the various temperatures above mentioned on two normal males and two with artificial fever in a room temperature of 24.4 to 27°C. and then after placing in a tent through which iced air was being pumped with an interior temperature of 11.7°C. Rectal and oral temperatures dropped appreciably and the temperature of the blood in the precubital vein fell in spite of the fact that the exposed arm had been kept at room temperature. The effect of rapid and deep respiration was to cause a further temperature drop. Only the head and upper chest were included in the cooled tent and the skin temperature of the remainder of the body remained nearly constant.

Some studies on the clotting time of rabbit blood following intravenous administration of ricin. HEWELL C. SAMUEL (by invitation) and EMMETT B. CARMICHAEL. Physiological Chemistry Department, School of Medicine, University of Alabama. (Read by title.)

The ricin was dissolved in physiologic salt solution (2.5 to 5 mgm. per cc.). The quantities of toxin injected per kilogram body-weight were 5, 7.5 or 10 mgm. and each dose was administered during about one minute. These injections varied in toxicity from 250 to 500 M. L. D. Twelve animals were injected and eleven control animals were tested for clotting time.

A modification of the capillary tube-horse hair method of Vierordt was used for determining the clotting time. The ear is treated with a depilatory and the blood is obtained from a marginal vein. The first drop is wiped off and a sample is taken only if the second drop is formed with a free rapid flow. The blood is allowed to flow immediately into the tube. The tube is placed in one of the creases of the palm to insure a fairly constant temperature. The white hair is pulled out a few millimeters at intervals of 5 to 10 seconds after the first 30 to 45 seconds. Clotting is evidenced by a red coagulum on the hair. The time is noted when the

second drop appears and the coagulation time is from this instant until the red coagulum is observed on the hair. The normal clotting time was determined for each animal before it was injected.

With the small dose it is usually possible to obtain a sample soon after the injection but with large doses, the blood pressure is so affected that several minutes may elapse before a free flowing drop appears. The clotting time seems to be slightly increased soon after the injection. This increase is followed by a definite decrease after about 30 to 60 minutes. The clotting time is always increased before death which occurs in about 4 to 8 hours. The maximum increase in clotting time varied from about 100 to 400 per cent for the different animals.

The clotting times for the controls remained fairly constant for several hours and never showed a marked variation.

Recovery heat production of caffeinized frog muscles. G. SASLOW (introduced by McK. Cattell). Department of Physiology, Cornell University Medical College, New York City.

The recovery heat following twitches and short tetani has been measured by Hill's myothermic method. Caffeine concentrations (in Ringer) between 0.02 and 0.05 per cent were used; the time of exposure varied from 15 to 30 minutes and the measurements were made subsequently over several hours.

The resting heat was increased several times and the recovery heat production was decreased greatly, at all concentrations. The greatest decreases in recovery heat were observed in those muscles showing the greatest increases in resting heat production. Recovery heats as low as 0.01 (initial heat 1.0) were observed. Occasionally the resting heat rate became steady at a much lower level after a stimulus than prevailed just before it, but in such cases, even if all the heat produced on stimulation were measured from the new base line, the computed recovery heat remained close to zero.

The high resting heat rate in the caffeine poisoned muscle may be related to an increased lactic acid production (observed by Meyerhof at higher caffeine concentrations) and to the increased oxygen consumption reported by Fenn. In the light of Fenn's observation it is suggested that the absence of recovery heat does not necessarily indicate a failure of the oxidative mechanism but that the recovery heat may be obscured by the high resting rate. In caffeinized muscles a consequence of stimulation may be that the energy derived from the vigorously occurring exothermic processes (such as oxidations and lactic acid formation) and measured up to this moment as resting heat is now being used to drive endothermic reactions (for example, phosphocreatine resynthesis) so that the muscles apparently fail to produce heat after the contraction or even appear to be absorbing heat for some time after the contraction. The magnitude of the recovery heat would thus depend upon the extent to which this transformation occurred.

A thermostromuhr operating on storage battery current. CARL F. SCHMIDT and ARTHUR M. WALKER. Laboratory of Pharmacology, University of Pennsylvania. (Demonstration.)

The method already described (Proc. Soc. Exper. Biol. and Med. **33:**

346, 1935) was shown in operation on an excised carotid artery. Representative calibration curves were displayed. Charts were shown illustrating the successful application of the method to the renal and coronary vessels of unanesthetized animals.

The absence of gastro-duodenal ulceration after prolonged physiologic hypersecretion. C. R. SCHMIDT and S. J. FOGELSON (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School.

Prolonged hypersecretion obtained by sham feeding 10 esophagotomized dogs three times daily for periods of 20 to 102 days produced no evidence of gastric or duodenal ulcers. Following the sham feedings, which lasted from 30 to 60 minutes, the gastric acidity varied from 90 to 120 clinical units free acid for the first two hours and from 60 to 90 clinical units for the following two hours.

Five dogs, on a sub-maintenance diet for 20, 22, 50, 60 and 60 days, showed progressive weight loss (10 to 40 per cent original body weight), progressive secondary anemia and compensated acidosis, and, at autopsy, a moderate duodenitis with no evidence of ulceration. Evidence of gastric hemorrhage appeared on the 35th and 40th days in two of these animals.

Five additional dogs were kept on a maintenance diet for 72 to 102 days. After the 50th day 300 cc. 0.35 per cent HCl were administered by stomach tube twice daily in addition to the three sham feedings. These animals showed a 4 to 10 per cent weight loss, slightly lowered CO_2 combining power, and in 3 of the 5 dogs a moderate non-progressive secondary anemia. At autopsy the gastric and duodenal mucosa appeared normal except for a moderate duodenitis. Two of the dogs showed signs of gastric bleeding between the 65th and 70th days during which time the strength of the acid gabage was increased to 0.5 per cent HCl.

Partial neutralization of the gastric contents (to an average of 60 clinical units free acid) occurred within 2 to 3 hours; in the majority of instances this resulted from regurgitation of intestinal juices (bile frequently present in gastric contents), and in a minority of instances from continued secretion of gastric juice high in neutral chlorides.

These observations suggest that a hypersecretion secondary to sham feeding in esophagotomized dogs over a period of 100 days even though augmented by the addition of 300 cc. 0.35 per cent HCl is not sufficient to cause gastro-duodenal ulceration.

The rate of removal of bile salts from the blood as affected by thyroxine. L. H. SCHMIDT (introduced by Shiro Tashiro). The Christ Hospital Research Institute and the Department of Biochemistry, University of Cincinnati, Cincinnati, Ohio.

Ingestion or injection of thyroxine produces a marked increase in the toxicity of certain bile salts for both guinea pigs and rabbits. It has been suggested that this increase in toxicity is due to a decrease in the capacity of the liver to remove bile salts added to the blood.

The present experiments show that following injection of thyroxine there is a pronounced delay in the rate of removal of cholic acid from the circulation of the rabbit. Five minutes after the timed intravenous injection of 0.3 gram of sodium cholate per kilogram of body weight, the blood

of normal control rabbits contains from 50 to 80 mgm. of cholic acid per 100 cc. of plasma; whereas the blood of the rabbits receiving thyroxine injections contains 80 to 125 mgm. of cholic acid per 100 cc. of plasma. This delay in the removal of cholic acid is even more marked 30 minutes after the sodium cholate injections; at this time the blood plasma of the normal rabbits contains only 2 to 9 mgm. of cholic acid, whereas the blood plasma of the rabbits injected with thyroxine contains 15 to 30 mgm. per 100 cc.

It is probable that the effect of thyroxine on the removal of cholic acid from the circulation is due to a disturbance of the capacity of the liver, either to destroy cholic acid, or to secrete it into the bile. It is interesting to note, however, that the livers of the animals that have received thyroxine do not show pathological changes.

Quantitative studies on nerve birefringence. FRANCIS O. SCHMITT and RICHARD S. BEAR (by invitation). Department of Zoology, Washington University, St. Louis.

Qualitative studies on nerve birefringence long ago established the fact that the optic axes of the myelin sheath micelles are radially oriented. Lack of quantitative data, however, seriously limits the application of polarization optics to the study of the relation between nerve ultrastructure and function. The present work supplies the basis for quantitative work.

The birefringence may be determined as an intrinsic property either of whole nerves or of single axon components (e.g. myelin sheath). Experimental difficulties in obtaining accuracy and reproducibility eliminated the use of freely suspended bundles of fibers. Compression of nerves between glass plates permitted accurate measurement but also produced phenomena which are doubtless artifacts. As the nerve is gradually compressed between the plates the retardation decreases faster than the decreasing thickness would warrant (confirming v. Ebner). Decompression increases the retardation to new high levels, as though the previous compression had further perfected the myelin radial orientation. Oscillographic studies of the action potential waves under these conditions revealed no changes associable with these large fluctuations in apparent birefringence. Compression experiments on single axons prove that much of the above behavior is explicable on the basis of deformation of axons which when injured present a greater fraction of their myelin elements in an orientation capable of producing retardation. For these reasons it is doubtful if any method employing nerve bundles can give sufficient accuracy and reproducibility for the present purpose.

A method was therefore evolved for determining the birefringence of the myelin sheath itself from observations on single axons. A mathematical expression was derived which permits calculation of the actual birefringence of sheath elements from measurements of sheath retardation and of axis cylinder and sheath dimensions. The average of observations on a number of axons teased from frog sciatics and spinal roots places the birefringence at 0.012 for the elements of the myelin sheath. The birefringence of whole nerves is an order of magnitude weaker, being slightly less than that of muscle (0.0025). The optical evidence is thus in agreement with x-ray diffraction conclusions as to the unusually high degree of organization of the myelin sheath.

The effect of exposure to cold upon the basal metabolism of the rat. EDWARD L. SCHWABE (by invitation) and FRED R. GRIFFITH, JR. Department of Physiology, Medical School, University of Buffalo.

The basal oxygen consumption of mature, albino rats subjected to low temperatures for varying periods of time was found to be considerably higher than that of litter mate controls kept in a constant warm environment.

Tissue gas studies in respiratory and circulatory disease. M. H. SEEVERS, H. R. HATHAWAY (by invitation) and R. T. STORMONT (by invitation). University of Wisconsin.

The gas depot method (Campbell) has been investigated as to its suitability for the determination of the partial pressures of O_2 and CO_2 existing in the subcutaneous tissues of the anterior abdominal wall of patients with respiratory and circulatory diseases.

Daily estimations in twenty patients over periods as long as six weeks, indicate that the initial nitrogen injection produces an inflammatory reaction of five to seven days duration, characterized as in normal subjects by high O_2 and low CO_2 values. Subsequently, exclusive of daily variation, the pressures remain relatively constant. In the present experience, deductions attempted from the data of an isolated determination are not trustworthy, since the daily variation is of sufficient magnitude that a representative result is not obtained. Curves plotted from the results of a series of daily analyses over a period of time reduce the probability of error and offer a means of study of the effects of therapeutic procedures.

Average pressures in this region for normal subjects were previously found to be CO_2 45 mm., O_2 22 mm. The variations found in disease are indicated by the following cases: Emphysema and cardiac decompensation, CO_2 84 mm., O_2 14 mm.; Coarctation of the aorta, CO_2 65 mm., O_2 4 mm.; Asthma (acute attack), CO_2 79 mm., O_2 15 mm.; Tracheal obstruction (carcinoma of thyroid), CO_2 65 mm., O_2 8 mm. Relief of the obstruction in the latter case by thyroidectomy was followed by a gradual return over ten days to the relatively normal level of CO_2 48 mm., O_2 19 mm.

Whereas the method appears to be of considerable value for studies of the peripheral circulation, it lacks the simplicity required for use as a general diagnostic procedure (Meyer, Klin. Wchnschr. **14:** 627, 1935).

An apparatus for the dry microanalysis of gases adapted to biological studies. M. H. SEEVERS and R. T. STORMONT (by invitation). University of Wisconsin. (Demonstration.)

An apparatus without stopcocks, based upon the principles of the Blacet-Leighton dry method (Ind. Eng. Chem. (Anal. ed.) **3:** 266, 1931) and incorporating the horizontal burette of Swearingen, Gerbes and Ellis (Ind. Eng. Chem. (Anal. ed.) **5:** 369, 1933) to facilitate manipulation, has been designed and adapted for biological studies. Improvements have been made in the methods of sampling, transport of samples, and introduction of the reagent into the gas. The mercury thread is activated by pressure transmitted through a steel diaphragm, thus eliminating contact with rubber and reducing the possibility of leaks. A constant temperature burette jacket increases the accuracy of the method. Four analyses may be in progress simultaneously, thus conserving much of the time ordinarily consumed in absorption of the various constituents.

Thymus, adrenals and thyroid in the response of the organism to certain drugs. H. SELYE. McGill University, Montreal.

Experiments on male and female rats 2 to 3 months of age show that rapid involution of the thymus and adrenal enlargement is produced by certain operative injuries and drugs, particularly those (like handling of the intestines, bone fractures, extensive skin lesions, and injection of adrenalin or drugs which stimulate adrenalin secretion) which cause marked nervous disturbances. Such stimuli also produce thyroid hyperplasia in the guinea pig, although this is not marked in the rat. Probably the hypophysis produces thyreotropic and adrenotropic hormone as an immediate response to various damaging influences. The adrenal then in its turn produces a substance which causes thymus involution. Fasting, adrenalin (0.2 mgm. daily subcutaneously), morphine (0.2 grain daily subcutaneously), atropine (0.2 grain daily subcutaneously), and various operative injuries, all of which normally cause marked involution, have little or no effect on the thymus weight of hypophysectomized or adrenalectomized rats. The low resistance of adrenalectomized rats to adrenalin and other toxic substances is corrected by administration of either cortin (5 Swingle and Pfiffner dog units daily) or sodium chloride (0.9 per cent solution given instead of drinking water, ad lib.), but such treatment does not influence the inability of the thymus of adrenalectomized rats to undergo rapid involution. Slight involution is obtained, however, with 200 dog units of cortin daily. Involution in intoxications may therefore either be due to a sudden extremely marked increase in cortin production, or to a contaminating substance which becomes demonstrable only in very high concentrations of cortin. The adrenal and thyroid hyperplasia and thymus atrophy probably represent a defense reaction. After some time drugs lose this effect and then the thymus grows even above normal size in spite of continued daily administration of a dose which causes atrophy in non-pretreated animals.

Excretion of urea and creatinine in the dog in relation to rate of urine formation. JAMES A. SHANNON. Department of Physiology, New York University College of Medicine.

The present observations were made to examine in dogs the relations between the rate of glomerular filtration (creatinine clearance) and the rate of excretion of water, and the effect of these variables upon the urea clearance. There being no standard of reference by which the clearances of various individual dogs can be converted to a common value, the data from each dog have been treated separately. All experiments include a fair representation of urine flows over the entire physiological range. In the major portion of the work we have attempted to observe the changes in urea and creatinine clearances which accompany changes in urine flow (reflecting changes in the state of hydration of the animal), as opposed to changes induced by the actual administration of water itself. This has necessitated working at stationary or falling urine flows, since no period has been taken within one hour after the administration of water. The examination of clearances during increasing rates of urine formation is under investigation. The experiments were conducted so that the rate of water excretion should return, at some time during the observations, as near as possible to the initial state. The urea and creatinine clearances have been examined in over 500 periods in 6 dogs, some on high and others on low protein diet. In general there is no point on the curve relating

urea clearance to urine volume that can be designated as an "augmentation limit." The urea clearance decreases systematically, both absolutely and relative to the creatinine clearance, with decreasing urine flow throughout the entire range of the latter. There is essentially no change in the creatinine clearance throughout the ordinary experimental range of urine flows (between 0.5 cc. and 3 cc. per minute for a 15–20 kgm. dog). At flows lower than 0.5 cc. per minute the creatinine clearance is sometimes observed to fall, a phenomenon possibly related to dehydration of the animal. At flows above 3 cc. per minute there is usually a tendency for the creatinine clearance to increase slightly.

The circulation time of the blood in dogs as measured by ionization methods. Effects of various physicophysiologic agents and of drugs. CHARLES SHEARD, EARL C. MCCRACKEN (by invitation) and HIRAM E. ESSEX. Division of Biophysical Research and Division of Experimental Medicine, The Mayo Foundation and The Mayo Clinic, Rochester, Minnesota.

The circulation time of the blood between the left jugular vein and the right femoral artery lying in the adductor canal was measured by the injection of radium C and the detection of its arrival in the femoral artery by ionization methods (Geiger counter). Investigations on the effects of various drugs and physicophysiologic agents show that the circulation time was decreased under ether anesthesia, following the injection of histamine or thyroxin, after exercise, after chilling to the degree productive of shivering, after exposure to an experimental temperature sufficient to elevate the rectal temperature, and during digestion. The circulation time was increased after the injection of nicotine, adrenalin, cobefrin, pituitrin and pitressin. The number of heart beats per basic circulation time was found to be quite constant irrespective of circulation time. In general, physicophysiologic agents (heat, cold, exercise or food) produced little if any effect on the number of heart beats per circulation time, whereas the administration of drugs produced increases in the number of heart beats.

The respiratory metabolism and lactic acid cycle in the excised skeletal muscle of the depancreatized-hypophysectomized dog. EPHRAIM SHORR, HENRY B. RICHARDSON and JOSHUA E. SWEET. Departments of Medicine and Experimental Surgery, Cornell University Medical College and The New York Hospital, New York City.

Previous reports from this laboratory (Am. J. Physiol. **97:** 1931; **101**, 1932) have dealt with the lactic acid oxidative quotient of excised skeletal muscle from normal and depancreatised dogs. The ratio $\frac{\text{mols lactic acid disappearing}}{\text{mols lactic acid oxidized}}$ was found to range from 2.0 to 7.2:1 with normal dog muscle, indicating a capacity to resynthesize added lactate. With muscle from diabetic dogs however, there was a loss of this function, to judge from the quotients of 1:1. In view of the influence of hypophysectomy on many aspects of pancreatic diabetes, it was of interest to extend the study of the lactic acid cycle to this type of animal.

Method. The hypophyses were removed prior to pancreatectomy. Seven experiments were carried out on 4 such animals, all of whom remained sugar-free in the fasting state. The gaseous and chemical studies were made by the technics previously described (ibid.).

Results. These are best analyzed by comparison with the previous findings using muscle of normal and depancreatized dogs. It is evident that the respiratory quotient of the Houssay animal is higher than that of the dog from which the pancreas alone has been removed. The lactate ion also stimulates respiration to a much greater degree. The lactic acid oxidative quotients indicate the preservation of the capacity to resynthesize added lactate, in contrast to depancreatized animals. In all these respects, the *in vitro* behaviour of the muscles of the Houssay dog resembles that of the normal, rather than the diabetic animal.

TABLE 1

Comparison of respiratory data and lactic acid oxidative quotients of normal, depancreatized and Houssay dogs obtained with washed muscle strips

TYPE OF ANIMAL	NUMBER OF EXPERIMENTS	RESPIRATORY QUOTIENT		OXYGEN CONSUMPTION (2 HOUR PERIODS)			LACTIC ACID OXIDATIVE QUOTIENT
		Non-nutrient	With lactate	Non-nutrient	With lactate	Change	
				cc. per moist gram	*cc. per moist gram*	*per cent*	
Normal	7	0.94	0.91	0.46	0.66	+44	4.3
Hypophysectomy and pancreatectomy	7	0.80	0.86	0.39	0.51	+31	4.1
Pancreatic diabetes	5	0.73	0.78	0.49	0.57	+16	1.0

Adreno-pituitary interrelationships and renal secretion in the opossum.[1] H. SILVETTE[2] and S. W. BRITTON. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

The average daily excretion per kilo body weight of 5 normal female opossums which were fed 100 cc. of 0.9 per cent sodium chloride p. k. b. w. each day was as follows: water, 76 cc.; sodium, 345 mgm. and chlorides, 556 mgm. (average of 15 daily runs). This represents total excretion of ingested salt, but (temporary) retention of about one-fourth of the ingested water. From five to twenty days following adrenal removal the same animals under uniform experimental conditions excreted on the average 66 cc. of water, 254 mgm. of sodium and 413 mgm. of chlorides daily (average of 18 daily runs). These 25 per cent diminutions in sodium and chloride excretion in adrenal insufficiency are correlated with previously reported increases in serum sodium and chloride concentration in adrenalectomized opossums.

The injection of post-pituitary liquid (Squibb) into adrenalectomized opossums which were studied over consecutive three-day periods (2 cases) notably increased the urinary output of sodium and chloride and brought about restoration of the normal (pre-operative) excretion of these substances. An important interrelationship between the posterior lobe of the hypophysis and the adrenal cortex, suggested by these observations, is being further investigated.

[1] Aided by a grant from the Rockefeller Foundation.
[2] E. R. Squibb and Sons Fellow in Physiology.

Saline and glucose effects on the survival of adrenalectomized opossums.[1] H. SILVETTE,[2] S. W. BRITTON and R. KLINE (by invitation). Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

In a recent report from this Laboratory it was shown that in the opossum (as well as the marmot) there occurred rises in serum sodium chloride following adrenal removal. This was in marked contrast to diminutions observed in the commoner laboratory animal types, e.g., dog, cat, rabbit, guinea-pig and rat. In view of the observed influence of salt and glucose solutions in prolonging the survival of adrenalectomized dogs and cats, it seemed important to determine the effect of such solutions on the life-span of the adrenalectomized opossum. In one series of animals given saline solution by mouth (100 cc. 2 per cent sodium chloride per kilo body weight per day), the average survival period was 52 days, or three times the average survival period of untreated winter-operated opossums.

Glucose solutions by mouth (100 cc. 5 per cent p. k. b. w.) were found to be effective in increasing the post-operative life-span approximately 50 per cent above that of untreated controls. In other control cases in which water in similar quantities was given by stomach tube, the survival period was not extended beyond the usual observed limits.

The results indicate that saline solutions exert merely a general beneficial effect on animals suffering from adrenal insufficiency. Thus, such fluids would hardly operate in the same way (to prolong life) in cases of salt-depletion (dog, etc.) as well as in salt-repletion (opossum).

The effect of anoxemia on the impermeability of the stomach to water. CLARK K. SLEETH (by invitation) and EDWARD J. VAN LIERE. Department of Physiology, University of West Virginia, Morgantown.

Adult, barbitalized dogs and cats were used in this work. Two animals were chosen, as near the same weight and age as possible. One served as a control, while the other was subjected to anoxemia.

The abdomen was opened, and a ligature was placed around the cardiac end of the stomach, leaving the blood supply intact. Although the animals had been starved for 48 hours the stomach was washed out with 5.0 per cent glucose, to remove any débris. All fluid thus used was carefully recovered. The pylorus was then ligated, again taking care to preserve the blood supply.

The stomach was filled with distilled water at body temperature by means of a large hypodermic syringe. Care was used to avoid distension. The abdomen was closed and the animal was kept warm. At the end of an hour the stomach was removed and its contents carefully measured.

The other animal was treated in the same manner, except that after the operative procedures the animal was placed in a steel respiratory chamber from which the air was exhausted to the desired degree of anoxemia. A constant stream of air provided adequate ventilation.

In 30 normal animals an average absorption of 4.41 per cent was found.

In 6 animals subjected to 12.25 per cent oxygen there was an average of 4.0 per cent absorption. In 9 animals at 10.5 per cent oxygen there was an average absorption of 3.8 per cent. Eight animals at 8.3 per cent

[1] Aided by a grant from the Rockefeller Foundation.

[2] E. R. Squibb and Sons Fellow in Physiology.

oxygen averaged 2.9 per cent absorption while 4 animals at 7.0 per cent oxygen showed an average absorption of 2.3 per cent. Higher degrees of anoxemia were not used, as some barbitalized animals could not withstand such severe anoxemia for an hour.

Since it is felt that all the figures obtained fall well within the range of experimental error, no importance is attached to the apparent variation present.

It seems that even extremely low oxygen tensions do not render the stomach permeable to water. Therefore, the difference in absorption between the stomach and the small intestine or the colon cannot be explained upon the basis of a greater venosity of the blood in the latter two.

The effect of accelerator nerve stimulation and of adrenalin on recovery from ventricular fibrillation in the cat. DIETRICH C. SMITH and ARTHUR G. MULDER (introduced by W. R. Amberson). Department of Physiology, College of Medicine, University of Tennessee.

The length of time for spontaneous recovery of the cat heart from ventricular fibrillation, produced by a two second faradization of the ventricles, was noted under control conditions and while the accelerator nerves were being simultaneously stimulated. It was found that accelerator stimulation shortened the time of fibrillation 60 per cent on the average. Hearts under the influence of adrenalin recovered from fibrillation immediately upon cessation of faradization of the ventricle.

It was also found that the time of fibrillation varies directly with the weight of the cat (size of the heart).

Cell changes induced in the hypophysis of the albino rat by repeated exposure to illuminating gas. ERMA SMITH and A. D. PICKETT (by invitation). Iowa State College.

Male albino rats exposed daily in a closed circuit respiration apparatus to 1.42 per cent gas-air mixture (0.36 per cent CO) for 3 to 4 months become sterile. The testes decrease in weight and the sperm become few and non-motile.

The pars anterior of the hypophysis of such rats undergoes cell changes which closely resemble castration effects. The basophiles (beta cells) increase in size and number and large, ring-shaped, colloid containing, vacuoles displace the nuclei to the periphery of the cells. The acidophiles (alpha cells) become fewer. The blood channels become dilated and engorged with blood.

The general condition of the rats bespeaks premature senility.

The postoperative effects of removal of the occipital cortex upon visual intensity discrimination in the cat. KARL U. SMITH (introduced by C. H. Graham) Brown University.

The present paper records experiments concerning the nature of intensity discrimination in the cat following removal of the visual areas of the cortex.

In one series of experiments, measurements of the threshold of discrimination of two lights were made at two differential intensity levels under conditions of general low illumination (intensity of the surround), the standard intensities at the two levels approximating respectively 0.5 and 50.0 apparent foot-candles. Measurements at the low and at the high

differential intensity levels on five animals, four with partial lesions of graduated size and one with complete removal of the striate cortices, gave small but insignificant indications of threshold loss, as compared with similar measurements made before the operations, but no differences in degree of threshold loss were ascertained at the two intensity levels.

In a second series of experiments the two differential stimuli were kept at constant values (approximately 0.5 and 50.0 apparent foot-candles) and the effects of complete destruction of the striate areas investigated under three different conditions of general illumination (intensity surround of the differential stimuli). A low (minimum) level of general illumination, a median level of general illumination, and a high level of general illumination were employed. Three animals, trained in the discrimination habit under all three conditions of general illumination prior to the operations, relearned the discrimination of the stimuli under the low conditions of general illumination after the operations. In comparison, after the operations two animals failed to relearn the second and all three failed to relearn the third habit after the operations. One control animal, with complete removal of the visual cortex on one side only, quickly relearned the discrimination of the two lights under all three conditions of general illumination after the operation. The significance of the results to a theory of the functions of the visual cortex is indicated.

The mechanism of the poisoning action of phlorizin on the kidney. PAUL W. SMITH. Department of Physiology, University of Illinois, Chicago.

In view of the fact that phlorizin abolishes the power of the kidney tubules to reabsorb glucose, and since this ability is related to the hexose-phosphate esterification mechanism, as is indicated by the observations of Wilbrandt and Laszt, Lundsgaard, Wertheimer, and others, it would seem that phlorizin might act by one of several mechanisms: it might abolish the esterification process, its major effect might be upon the ester hydrolysing reaction, or both might be equally inhibited. In the first case the hexose monophosphate content of the phlorizinized kidney would be expected to be low; in the second case, elevated. Actual measurements of the hexose monophosphate content of normal and phlorizinized kidneys have shown essentially identical values, suggesting with regard to this ester that both formation and breakdown, if inhibited, must be affected equally.

Fatigue, contracture and rigor mortis in the frog's gastrocnemius. WILLIE W. SMITH (by invitation) and F. H. PIKE. Department of Physiology, Columbia University.

There are certain facts, derived from the literature and our own observations: 1. Contracture occurs at a certain stage in the control muscle when there is a supply of oxygen in the nutrient fluid (Ringer). This contracture increases as the experiment continues until neither contraction nor relaxation occurs. Several hours rest in presence of oxygen brings on relaxation, after which the muscle will contract for a brief interval.

2. Contracture and more or less permanent shortening, considering comparable states, more often than not comes on earlier in Ca-free Ringer than in control preparations.

3. Rigor mortis will develop in presence of mono-iodo-acetic acid.

4. Rigor mortis develops more rapidly in muscles in situ whose afferent

and efferent nerve connections with the central system are intact than when these connections are interrupted.

5. Rigor mortis does not develop in excised mammalian muscles in Ringer when the pressure of oxygen is high.

A theory is developed to show that contracture, fatigue and rigor mortis result from the failure of sufficient resynthesis of lactic acid to glycogen in the relaxation phase of muscular activity due to 1, virtual exhaustion of all reserve material in the muscle where the concentration of oxygen is sufficient, or 2, the failure of resynthesis, where *a* the oxygen pressure is insufficient, or *b*, where carbohydrate is not broken down to lactic acid in the presence of mono-iodo-acetic acid.

Contracture in the gastrocnemius of the frog. Calcium-free Ringer contracture and the effects of various substances upon it. WILLIE W. SMITH (introduced by F. H. Pike). Department of Physiology, Columbia University. (Read by title.)

The perfused gastrocnemius was set up in the perfusate in series with the control; maximal stimuli, such as one cycle from a 60 cycle alternating current, passing through the solution once a second. Isotonic contractions were recorded.

Contracture was studied in 200 frogs throughout the year. The highest recorded was 163 per cent of the initial contraction height; the highest persisting contracture, 145 per cent. In these, return to normal length occurred only after several hours, even if $CaCl_2$ is added. 28 mgm. to 42 mgm. per cent of KCl, sufficient concentrations of $BaCl_2$, $SrCl_2$, $MgCl_2$, or methylene blue will prevent development of this contracture.

The highest contracture is transitory, reappearing only after rest. Reappearance may be prevented by these substances which, themselves, lead to a slowly developing contracture: mono-iodo-acetic acid, NaF, caffeine, chloroform, strong acid or alkali. Veratrin changes the picture to that typical of veratrin poisoning in the normal muscle, after the passing of which, the calcium-free Ringer contracture is not seen.

Acid at about pH 5 will cause the contracture to disappear when not extreme, unless the perfusate has been acid. At pH 10 or with 100 mgm. per cent of $NaHCO_3$, the contracture may gradually disappear.

Increasing NaH_2PO_4 to 20 mgm. per cent or omitting NaH_2PO_4, or KCl from the perfusate does not change the myogram.

Regulation of fetal respiration. FRANKLIN F. SNYDER and MORRIS ROSENFELD (introduced by E. K. Marshall, Jr.). Department of Obstetrics and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University.

Direct observation of fetuses at term was made in rabbits in which parturition was inhibited by injection of extract of urine of pregnancy. The uterus was exposed by laparotomy in a warm saline bath and fetal movements were studied through the transparent wall of the unopened uterus. Anesthesia was obtained by section of the spinal cord at the lumbar level. In the absence of a general anesthetic fetuses exhibited rhythmic excursions of the thorax and abdomen which were similar to post-natal respiratory movements. The rate varied, ranging up to sixty per minute.

Anoxemia depressed or inhibited fetal respiration. This was induced by administration to the mother of a gas mixture containing 4 per cent oxygen in nitrogen with and without the addition of 5 per cent carbon dioxide. Maternal respiration was stimulated by this mixture. Carbon dioxide excess obtained by administration to the mother of $7\frac{1}{2}$ per cent carbon dioxide in air and in oxygen failed to influence fetal respiration to a significant degree. Carbon dioxide deficit induced by artificial hyperventilation of the mother resulted in a slowing of respiration or apnea in the fetus. Intrauterine fetal respiration was thus readily depressed by anoxemia but was quite unresponsive to an excess of carbon dioxide. A certain minimal carbon dioxide tension appeared to be essential however for the maintenance of intrauterine respiratory activity.

Indispensability of the hypophysis to the Staub-Traugott phenomenon. S. SOSKIN, I. A. MIRSKY (by invitation), L. M. ZIMMERMAN (by invitation) and R. C. HELLER (by invitation). Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

It has been shown previously that, under the appropriate experimental conditions, repeated dextrose tolerance tests yield successively "better" tolerance curves (Staub-Traugott effect) in completely depancreatized dogs. This phenomenon, like the tolerance curve itself, cannot therefore be ascribed to the mobilization of extra insulin from pancreas.

We have accounted for the normal dextrose tolerance curve by postulating a homeostatic mechanism, whereby the liver decreases its supply of sugar to the blood in response to an influx of exogenous sugar. This is analogous to the regulation of room temperature by a thermostat-furnace arrangement. In this analogy the induced rise in the blood sugar level, like a rise in room temperature, is the stimulus for the regulating mechanism. The action of insulin may be compared to the setting of the thermostat, i.e., it is one of the factors which determines the blood sugar level (threshold) at which the homeostatic reaction occurs.

We have recently reported that normal dextrose tolerance tests may be obtained in completely hypophysectomized and depancreatized dogs maintained without insulin. This accords with our previous work and shows, moreover, that the hypophysis is not essential to the normal dextrose tolerance curve. It was concluded that the hypophysis is another factor (acting antagonistically to the pancreas) in the endocrine balance which sets the liver threshold.

In the present report it is shown that, while normal dextrose tolerance curves are obtained in hypophysectomized dogs, such animals do not yield the Staub-Traugott effect. It is suggested that the latter phenomenon, in normal animals, may be mediated by the hypophysis and be due to a lowering of the liver threshold. It may be regarded as a separate and slower adjusting mechanism than that which is responsible for the tolerance curve itself. Such a secondary regulation may represent a more efficient means by which the liver responds to a prolonged high carbohydrate environment. This would be analogous to a re-setting of the thermostat on the furnace to a lower level with the advent of summer. From a practical viewpoint, our results indicate that the Staub-Traugott effect may serve as an index of pituitary function.

Biochemical studies on the blood of dystrophic rabbits. HOWARD C. SPENCER (by invitation) and S. MORGULIS. Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.

In patients with muscular dystrophy a typical sugar tolerance curve has been found, characterized by a zigzag course. In rabbits, in which muscular dystrophy has been produced by means of the Goettsch-Pappenheimer diet 13, we failed to find this characteristic myopathic sugar tolerance curve. Studying the blood from the dystrophic rabbits we found that the values for fasting blood sugar, lactic acid and acid soluble P did not deviate appreciably from those of the control rabbits. In contrast to these constituents, the blood lipid phosphorus and the total blood cholesterol is definitely increased in the dystrophic animals. The increase in cholesterol runs parallel to the degree of muscle degeneration, as shown by simultaneous blood analysis and histological examination of the muscles, as well as by the study of the blood of rabbits approaching dystrophy and of those recovering from the disease. These results suggest a close relationship between nutritional muscular dystrophy and cholesterol metabolism.

Stimulation of the endogenous respiration of bakers' yeast by carbon monoxide. J. N. STANNARD (introduced by W. O. Fenn). Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

It has been found in these investigations that the oxygen consumption of "fully dissimilated," i.e., starved, bakers' yeast suspended in non-nutrient media is stimulated rather than inhibited by carbon monoxide mixtures containing 79 per cent CO and 21 per cent oxygen. The stimulation averaged 50 per cent of the rates measured in air and lasted as long as 5.5 hours. Blank experiments and calculations showed combination of the CO with the alkali in the side-arm of the respirometer bottle and chemical combination with the respiratory enzymes to be negligible fractions of the total.

Thinking that the phenomenon might be a burning of the CO similar to that observed by Fenn and Cobb in skeletal and cardiac muscle, measurements of the total volume of gas consumed were compared with the total volume of oxygen consumed using a slight modification of the method employed by these authors. The total gas consumed could be accounted for as oxygen. The stimulation is, therefore, an increase in oxygen consumption.

Stimulation by CO was observed only when the cells had low rates of respiration and presumably low concentrations of cellular reserves. Normal inhibitions ranging up to 60 per cent were obtained when ample stores were present. Stier and Stannard (J. Gen. Physiol. **19:** 461, 1936) have observed that the apparent R.Q. often decreased from the usual value of one to 0.75 to 0.8 in "fully dissimilated" yeast. In the present experiments a sudden lowering of the rate in air usually precedes the onset of stimulation. Furthermore, the stimulation seems to persist in the presence of 10^{-3} molar KCN. From these facts it is suggested that the stimulation by CO is associated with the oxidation of some substrate not normally utilized when ample stores of glycogen are available, the oxidation being mediated perhaps by a separate enzyme system.

Changes occurring in a circulation schema when failure of one or both sides of the heart is simulated. ISAAC STARR, JR. and ARTHUR J. RAWSON (by invitation). Research Dept. of Therapeutics and the Johnson Foundation, Univ. of Pennsylvania.

A circulation schema has been designed with pumps to represent each side of the heart, and with appropriate tubes and variable resistances between. These pumps have been constructed so that practically no sucking occurs during diastole, and also, so that the work applied increases as diastolic filling increases. The pumps, therefore, imitate the type of adaptation exemplified by Starling's "Law of the heart." By appropriate adjustments the strength of a pump may be changed either by shortening the stroke or weakening the force applied; in the latter case systole increases its length relative to diastole. Increases of rate may be likewise accompanied by increased relative length of systole. Records obtained from "arterial" and "venous" pressures roughly resemble those found in living animals. The work diagram of the pumps resembles that suggested for the heart by Straub.

When one "heart" is weakened, or rendered completely impotent, the resulting effect on the circulation varies greatly under different conditions. If the general systemic pressure (i.e., the pressure existing within the system when the heart is stopped) is high, one sided failure causes much less change than when this pressure is low.

When both sides of the "heart" are weakened together the "systemic venous pressure" rises little if at all. When one side fails, the rise in pressure in the adjacent "veins" varies with the strength of the "heart" which failed. Thus, after complete failure of the "left heart" the increase of pressure in the "pulmonary veins" is considerable; but after complete failure of the right "heart" the increase of "systemic venous pressure" is always very much smaller and is altogether absent under certain conditions.

A circulation schema, designed for the investigation of changes analogous to those occurring in failure of the heart and circulation. ISAAC STARR, JR. and ARTHUR J. RAWSON (by invitation). Research Dept. of Therapeutics and the Johnson Foundation, Univ. of Pennsylvania. (Demonstration.)

A method for studying the response of the colon to various stimuli, in particular, pitressin and pitocin. F. R. STEGGERDA and CESARE GIANTURCO (by invitation). Dept. of Physiology, University of Illinois, Urbana, and Carle Hospital Clinic, Urbana, Illinois.

The response of the colon in normal animals to intravenous injections of various compounds can be studied satisfactorily by making the walls of the colon opaque to x-rays by injections of thorium dioxide sol (thorotrast).

The thorotrast is injected in small quantities under the serosa directly into the walls of the colon. These injections are made at several points along the entire length of the colon. In 4 to 6 weeks after the operation, the thorotrast is uniformily distributed throughout the walls of the colon; and since it does not disappear by absorption, it presents an opaque colon for an indefinite period of time. The pictures of colons injected in this way eight months ago are still as clear as those taken shortly after the operation.

Intravenous injections of pitressin caused a marked increase in tone of the colon, showing a decrease in size both in length and diameter. The maximum contraction is reached in 15 to 20 minutes after which there is a gradual relaxation back to the normal size. Similar injections of pitocin have no apparent effect on the size of the colon.

Graded responses and conduction without apparent contraction. S. E. STEIMAN (by invitation) and F. H. PRATT. Department of Physiology, Boston University School of Medicine, and the Evans Memorial.

Conditions favoring local graded contractions in the muscle fiber were studied with controlled condenser discharges in the membrana basihyoidea of *Rana pipiens.* A fiber giving local contractions to a constant strength of stimulus can also respond in an all-or-none manner to the same stimulus. Upon continued stimulation, the latter contractions may disappear, leaving only local responses. However, if this strength of stimulus eliciting only local responses is increased in duration, the all-or-none effect reappears. These local contractions can also be fatigued.

If a fiber is responding locally, a threshold stimulus will produce contraction at some distance from the electrode, the portion of the fiber between electrode and responding area remaining quiescent. A slight increase in stimulus, however, produces visible response in the previously quiescent region.

Such a distant localized contraction can be obtained either with two stigmatic electrodes or with a stigmatic and a diffuse electrode. With the latter, the electrical field was changed by shifting the position of the diffuse electrode, or the direction of the current reversed by a pole changer. If the phenomenon were due to electrical conduction a change in the response on alteration of the electrical field would be expected. The response, however, was not affected by such changes.

If the electrode be moved along the quiescent region toward the area that is responding no change is produced. But if the electrode be placed at any other point, this specific contraction is eliminated and another distant localized response may be obtained.

By applying pressure to the quiescent region by means of a glass rod, these "distant" responses are abolished. If now the rod be slightly raised, the response returns. If this effect were due to electrical conduction, then shifting the electrode elsewhere at a comparable distance on the membrane, or compressing the quiescent region, should not alter the response. It is concluded, therefore, that the distant localized contractions are a result of physiological conduction, and that in these single muscle fibers we may have conduction without apparent contraction.

The psychophysiology of pitch and loudness. S. S. STEVENS (introduced by H. Davis). Harvard University.

The psychophysiological problem in audition is that of discovering the physiological processes which underly the responses of living organisms to tonal stimuli. The study of the electrical potentials generated in the receptor mechanism and the auditory nerve provides us with knowledge of the functional relationships between certain aspects of the physiological system and the dimensions of the auditory stimulus. These functional relationships can then be compared with those obtained in psychological

experiments between stimulus and response (perception), and, if the relationships are the same in both cases, the presumption is that the peripheral physiological mechanism of auditory perception has been discovered.

Thus the correspondence between the threshold of hearing in human observers and the minimal intensity of sound necessary to elicit electrical responses in the cochleas of animals suggests that the electrical response is basic to hearing.

Pitch. The correspondence between the frequency of the stimulus and of the impulses in the auditory nerve must be considered irrelevant to the perception of pitch, because the correspondence breaks down at critical points. However, the regions of the basilar membrane which are resonant to different frequencies, determined by damaging the organ of Corti at various places and noting the effect on the electrical response, correspond with locations derived from an integration of recent data on differential sensitivity to pitch in human ears.

Loudness. In the organ of Corti equal magnitudes of electrical potential are generated by tones (of different frequency and intensity) which sound equally loud to human observers, i.e., equal potential contours correspond to equal loudness contours. Furthermore, the size of the electrical potential at a constant frequency is the same function of the intensity of the stimulus as is the subjectively estimated loudness of the tone, except for an overload effect which appears in the electrical response at high intensities.

As regards the auditory nerve, the notion that the total number of impulses passing along it determines the loudness of a tone must be modified in favor of the conception that loudness is due to the total number of active fibers.

The effect of various distending pressures on the activity of the dog's ileum.

MARGARET SUMWALT and HUGO KRUEGER. University of Michigan.

The recording system consisted of a Brodie bellows, connected with a water-filled balloon in a Thiry-Vella fistula of the dog's ileum. The pressure distending the balloon could be kept constant, or varied at will. In one series of experiments a constant pressure was applied for 200 minutes, and only varied from day to day. In the other, for each experiment, pressure was initially 5 cm. H_2O; it was increased in steps of 10 cm. every 10 minutes, 8 times; and then, after a half-hour's pause at the highest pressure of 85 cm. H_2O, descended by similar steps to the initial value.

The average frequency and amplitude of the rhythmic contractions, and the maximum relaxation (in terms of the volume of water in the balloon) were measured, and the work done by the intestinal musculature was calculated at each pressure. The way in which these quantities varied with pressure proved to be essentially the same, whether the pressure was applied for 200 minutes, or for 10 minutes after previously higher values.

The volume accommodated by the intestine increased along a roughly sigmoid curve, as the pressure increased. The frequency of rhythmic contractions was usually at a minimum at intermediate pressures; greater at extremes of pressure. The average amplitude of individual contractions was greatest at those intermediate pressures where their frequency was least. Work also showed a maximum at medium pressures, though this maximum occurred at a somewhat higher pressure than that for amplitude.

A rubber-celluloid capsule for measuring venous pressure. H. MORROW SWEENEY (by invitation) and H. S. MAYERSON. Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans. (Demonstration.)

A rubber-celluloid capsule is presented as an improvement over those previously described for ascertaining venous pressure. The outstanding advantages are 1, air-tight skin contacts can easily be made on smooth or irregular surfaces which will withstand the highest pathological venous pressure; 2, there is a minimum of skin distortion; 3, no special lighting is required for good visibility; 4, the construction is simple and inexpensive. Readings obtained with this capsule have been checked by simultaneous direct puncture measurements and found to agree within one centimeter of water.

Synchronism of heart and respiratory beat in Limulus. JOHN TAIT and N. J. BERRILL (by invitation). McGill University, Montreal.

On *a priori* grounds it was conjectured that the beat of the respiratory appendages of Limulus may synchronize with the heart beat. When arrangements are made to observe both simultaneously, it transpires that when the respiratory appendages do move they do so in precise *tempo* with the heart beats. As the individual leaves of the gill-books likewise increase in volume with each opening movement of the respiratory appendages, this rhythmic volume change derives presumably from rhythmic changes of blood pressure. Because the heart of this arachnid thus constitutes an essential part of its "outer motor mechanism" of respiration, one can better understand 1, the reason for the neurogenic control of the Limulus heart, as discovered by Carlson; 2, Plateau's failure to discover externally visible respiratory movements in scorpions and spiders; 3, the curious retention of the "book" form of respiratory organs in terrestrial arachnids.

Apparatus used in the study of colon motility. R. D. TEMPLETON, W. F. BOLLENS (by invitation), H. C. LAWSON, A. LUTZ (by invitation), E. L. BORKON (by invitation) and E. A. GALAPEAUX (by invitation). Department of Physiology, University of Chicago and Loyola University School of Medicine. (Demonstration.)

The tandem balloon set consists of three rubber tubes sealed together so as to form a unit of three compartments and strengthened with coiled springs made of music wire (J. Lab. and Clin. Med. **15:** 3).

The manometer clamp which is made of brass is capable of holding six manometers in two rows of three each. The close relation of the manometers to one another makes it possible to record from them simultaneously without excessively long riders on the floats (J. Lab. and Clin. Med. **17:** 2).

The manometer cap is $3\frac{1}{2}$ cm. long and 3 mm. larger in diameter than the manometers. A thin copper plate bent at three sharp angles is inserted into the cap to cause a tight fit. A brass arm is soldered to the cap 1 cm. below its top and extends laterally 1 cm. To the free end of this side arm is soldered a coiled spring, made of no. 26 brass wire, 2 mm. in diameter and $2\frac{1}{2}$ cm. long. This coiled spring extending upward has fastened, by collodion, to its free end a piece of drawn glass. The cap can be rotated upon the manometer and the drawn glass thus brought against the rider to the desired pressure.

The float employed is open-bottomed and can be sunk to a desired level by introducing the amount of manometer fluid necessary.

The rider is fastened to the upright part of the float with a cross shaped strip of perforated celluloid. Through the perforations adjustments of the rider in length and level are easy.

The manometer stand is fastened to the table in such a way that the upright arm to which the manometer clamp is fastened is about 14 cm. beyond the edge of the table. This allows the manometers to extend below the level of the table. Joints in the manometer stand make easy the adjustment of the manometer set to the kymograph.

A study of pyloric function during gastric emptying of oil and hydrochloric acid solution. J. EARL THOMAS and J. O. CRIDER. Jefferson Medical College.

In spite of experimental proof from numerous sources that pyloroplasty, pylorectomy or gastroenterostomy does not shorten the emptying time of the normal stomach, various authors continue to attribute the delayed emptying of fats and HCl solutions to closure of the pylorus. An investigation is now in progress designed to determine whether the pylorus has any demonstrable influence on the rate of emptying of liquids including various substances known to delay gastric emptying.

Observations have been made on two dogs provided with cannulated gastric and duodenal fistulas. The emptying times of normal saline, N/20 HCl and olive oil have been determined under the following conditions: 1, without apparatus (normal controls); 2, with an apparatus in the pylorus which maintains a 7 mm. opening between the stomach and duodenum, and 3, with an apparatus identical to the other externally but which does not open the pylorus (dummy controls). These observations are being supplemented by graphic records of pyloric activity.

Maintaining a free communication between the stomach and duodenum does not shorten the emptying time of any of the liquids investigated. On the contrary, keeping the pylorus open appears to delay somewhat the emptying of oil. This is, perhaps, to be attributed to greater regurgitation from the duodenum.

Graphic records show that the principal effect of fat on the pylorus is inhibition of the rhythmic contractions coincident with inhibition of gastric peristalsis. The effects of fat on pyloric tonus are slight, variable, and probably of little consequence.

Parathyroidectomy in the fowl. D. L. THOMSON, C. M. HARLOW (by invitation), L. I. PUGSLEY (by invitation) and H. SELYE. McGill University, Montreal.

Parathyroidectomy was successfully performed upon 10 cocks of various breeds; the serum calcium fell from an average of 11.0 mg. per cent to 7.3 in 24 hours. Three of these birds displayed tetany and died within a week; in the others, the serum calcium had regained normal levels 7 days after operation. In a group of 6 non-laying hens the serum calcium fell in the first 24 hours from 11.5 to 8.2 mgm. per cent, but all recovered: in another group of 5 hens which were in poor nutritional condition, however, 4 died in the first four days after operation. In 5 laying hens, the serum calcium fell from 24.2 to 9.2 mgm. per cent in the first 12 hours; one died on the

third day. It appears that the abnormal elevation of the serum calcium in laying hens is dependent on the integrity of the parathyroids. Parathyroid extract was administered to birds parathyroidectomized 30 days previously, in 5 hourly doses of 20 units each: the serum calcium rose in 10 hours from 10.8 to 13.2 in one case (male) and from 11.4 to 14.6 in another (female). The plasma inorganic phosphate tends to vary inversely with the serum calcium fluctuations. Accessory parathyroid tissue has not been found in any of the birds examined.

Extrapyramidal activity of the cat's cerebral cortex: motor and inhibitory. SARAH S. TOWER. Department of Anatomy, Johns Hopkins University.

By severing the medullary pyramids, pyramidal and extrapyramidal functions of the cerebral cortex may be dissociated. Cortico-spinal activity is thus interrupted whereas the extrapyramidal activities survive and may be studied by electrical stimulation of the cortex. Utilizing this procedure the extrapyramidal functions of the cat's cortex have been investigated, and found to include both motor and inhibitory actions.

Inhibition may be brought to bear from the cerebral cortex on tonic contraction in skeletal muscle, and on movements rhythmically recurring. Relaxation of tonix contraction has been obtained only from the pre- and post-cruciate gyri. Within this limited field two varieties of action have been discriminated, a weaker contralateral action, seemingly co-extensive with Betz cell distribution in the cortex, and a stronger action, chiefly ipsilateral, from the border zone adjacent to the gyrus proreus. Repetitive movements such as running, clawing or striking, nystagmus, licking and chewing, breathing and tail wagging have been slowed and stopped, temporarily or permanently, by stimulating either the gyrus proreus, or over a large field in the sylvian region. During such stimulation the animal appeared fixed at attention, eyes front, head raised, forelegs extended, hindlegs extended or semiflexed, tail out straight.

The extrapyramidal motor activities of the cortex include the usual adversive mechanisms, frontal and occipital for the eyes, temporal for the ears, and superior parietal for head and trunk. Moreover, each of these may also evoke complex purposive movements of the extremities. Besides these the peri-cruciate region presents several overlapping motor fields, a narrow field antero-medially for striking or placing movements primarily of the crossed foreleg; a larger field lateral to this for a flexor-extensor sequence in one or both forelegs reproducing the nursing movements of kittens and the reaction of pleasure of adult cats; and a still larger field, perhaps the entire area, for diagonal progression, which begins in the crossed fore- or hindleg according as the stimulation is anterior or posterior to the cruciate sulcus.

Meagre evidence has also been collected concerning the subcortical course of the projection systems involved, and the brain stem centers upon which these operate.

Serum protein of fishes: colloid osmotic pressure and other data. ABBY H. TURNER. Bergens Museum Biologiske Stasjon, Herdla, Norway, and the Physiological Laboratory, Mount Holyoke College.

Colloid osmotic pressure determinations on the serum of marine fishes were reported last year from studies at Woods Hole on a limited number

of teleost and elasmobranch species. On the Norwegian coast it was possible to extend considerably the range of species investigated. These additional findings give a more secure basis for considering the significance of serum protein in fishes.

Forty-seven individuals representing 17 species were used, 10 teleost species, 6 elasmobranchs, one cyclostome. Measurements were made of the colloid osmotic pressure, the total nitrogen and non-protein nitrogen of the serum, and of the refractive indices of both serum and its ultrafiltrate. The nitrogen analyses were made by Can. Mag. Francis Wolff at the Fiskeriforsöksstasjon in Bergen.

Of the ten teleosts, three, Anguilla (eel), Scomber (mackerel), and Belone (hornfish) gave high c.o.p. readings, averaging from 166 to 193 mm. water pressure. All are vigorous fish with a wide range and of firm, non-watery flesh. Anarhichas (wolffish) gave 151 mm. Of four Gadids (cod and allies) three, including two from a depth of 300 metres, were of middle range, 113, 116, 154 mm. c.o.p. while the fourth, a pollack, had a pressure of only 80 mm. Cyclopterus (lumpfish) and Lophius (goosefish) gave the low figures, 81 and 44 mm. respectively. Both are relatively phlegmatic and of watery flesh, Lophius especially.

The refractive indices and protein nitrogen findings showed a general though not a close correspondence with the c.o.p. The range of protein percentage was from 2.50 (Lophius) to 5.20 (Scomber).

All elasmobranchs were low like those reported last year, with average c.o.p. from 14 to 28 mm. and with protein percentage from 1.00 (Spinax) to 3.22 (Raia fallonica). Five of the species were from the deep sea habitat.

One sample from several Myxine (hagfish) gave a c.o.p. of 24 mm. but appeared to have a relatively high protein percentage, 3.54.

The effect of anoxemia on the emptying time of the human stomach. EDWARD J. VAN LIERE, DONALD H. LOUGH (by invitation) and CLARK K. SLEETH (by invitation). Department of Physiology, University of West Virginia, Morgantown, W. Va.

Eight male medical students in good health were used in this work. They were given a test meal ta 8:30 a.m., having taken no food since the previous evening meal. This test meal consisted of 15 grams of Quaker Farina and 1 gram of salt, added to 350 cc. of water and cooked to a total volume of 200 cc. Fifty grams of barium sulphate were added, so that the emptying time of the stomach could be determined fluoroscopically.

Several normal determinations were made upon each subject. The subject was then placed in a specially constructed, steel respiratory chamber, capable of accommodating comfortably two or three individuals, and subjected to anoxemia. The anoxemia was produced by exhausting the air from the chamber at a rate faster than it was allowed to enter. By this means a uniform degree of anoxemia could be produced, and at the same time, adequate ventilation provided for the occupants of the chamber.

Air pressures of 560 mm., 515 mm. and 460 mm. of mercury were used in this experiment. These pressures correspond with oxygen percentages of approximately 15.37 per cent, 14.25 per cent and 12.28 per cent, respectively. These oxygen percentages correspond to respective altitudes of about 8,000 feet, 10,000 feet and 14,000 feet.

It was found that each of these degrees of anoxemia caused a delay in the emptying time of the stomach. In 3 subjects with determinations at 15.37 per cent oxygen there was an average delay of 8.5 per cent. In 7 subjects with determinations at 14.25 per cent oxygen the average delay was 13.9 per cent. Determinations were made on all subjects at 12.28 per cent oxygen, with an average delay of 54.1 per cent.

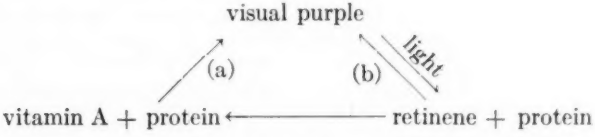
In view of other work it is felt that the delay may be caused by a vagospastic pylorospasm, as well as by a direct depression of the gastric musculature. Splanchnic stimulation could account, in part, for this depression. Such a stimulation might be a direct effect of the anoxemia, or it might be due to the increased secretion of epinephrine known to accompany anoxemia. Further, it is possible that the anoxemia, acting directly on the muscle, might depress gastric motility.

The movement of univalent cations against concentration gradients from small intestine to the blood in the presence of polyvalent ions. MAURICE B. VISSCHER and RAYMOND C. INGRAHAM (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

Previous studies have shown the effect of polyvalent anions upon the absorption of the univalent anions chloride and bromide, from the intestine. The fluid in the intestine becomes virtually chloride- and bromide-free in the presence of sulphate, citrate, phosphate and ferrocyanide. A comparable univalent ion impoverishment has now been found to occur in the case of cations when polyvalent cations are present. In particular, in the presence of magnesium salts, sodium is absorbed from the small intestine against large concentration gradients, approximately of the same magnitude as those found previously in the case of univalent anions in the presence of polyvalent ones. The mechanism of active absorption of this type will be discussed.

Sensory adaptation and chemistry of the retinal rods. GEORGE WALD and ANNA-BETTY CLARK (by invitation). Biological Laboratories of Harvard University, Cambridge.

Changes in concentration of visual purple in the retinal rods are reflected inversely in changes in the threshold to light, such as occur during light and dark adaptation. The reactions initiated by light in the rods are described partly by the equations:



The two distinct processes which form visual purple (*a* and *b*) should correspond with two modes of dark adaptation. The equations indicate that these should be separated partly by the following procedure: If a completely dark adapted eye containing visual purple alone is brightly illuminated for a few seconds, considerable retinene is formed, but very little vitamin A. The succeeding dark adaptation should consist preponderantly of reaction (b). If, however, a dark adapted eye is exposed to

light for a number of minutes, large amounts of retinene are converted to vitamin A, and the succeeding dark adaptation should consist to a greater extent of process (a).

Consistent with these predictions, it is observed that between a given initial and final threshold the rods may dark adapt along many different paths, depending upon the period of the preceding light adaptation. For example, after a ten second exposure to 1890 millilamberts, the dark adaptation of a small area 10° to the nasal side of the fixation point is complete in about 14 minutes. After 7 minutes exposure to 333 millilamberts, the initial threshold is about the same, but dark adaptation proceeds much more slowly, and is still incomplete after 35 minutes.

The transition from the fast to the slowest type of dark-adaptation requires about 10 minutes exposure to 333 millilamberts. This is the period required for the chemical cycle to attain a steady state at this intensity, the end-point of the light adaptation process.

Reticulocytogenic action of pernicious anemia urine extracts. ORVILLE S. WALTERS (introduced by A. B. Hertzman). Department of Physiology, St. Louis University School of Medicine.

In order to investigate the reticulocytogenic activity of the urine in pernicious anemia, samples of urine were collected prior to and during liver treatment from two patients in severe relapse who had received no antianemic therapy for several months.

Extracts of the urine were made by adsorption with charcoal (Norite) at pH 5.0 and eluting with 50 per cent ethyl alcohol, then concentrating to a low volume by vacuum distillation. These extracts were tested by the guinea pig method.

Extracts of urine collected both before and after the beginning of liver treatment were strongly reticulocytogenic when administered intraperitoneally, as were also similarly prepared extracts of normal urine.

Extracts made with 70 per cent alcohol were as effective as those prepared with 50 per cent. The reticulocytogenic effect of the extracts was not abolished by five minutes' boiling.

The function of interchanged fore limbs in salamanders. PAUL WEISS (introduced by R. W. Gerard). Department of Zoology, The University of Chicago.

Previous experiments by the author have suggested that a nerve becomes specified by its muscle in the sense that it can respond selectively to specific central impulses destined for that muscle. A nerve connected with a strange muscle undergoes a corresponding re-specification. This can be deduced from the phenomenon of "homologous response" observed in supernumerary transplanted muscles and limbs, i.e., the fact that a supernumerary muscle contracts always simultaneously with the *synonymous* muscle innervated from the same side of the cord. This phenomenon has been demonstrated to be based neither on specific nerve connections nor on sensory control.

A central discharge pattern would, then, consist of a regular sequence of specific activities each one engaging its corresponding set of specified motoneurones. Consequently, if a normal left limb is removed and a right limb

transplanted in its place, the latter being anatomically a mirror image of the former, and if the central discharge pattern continued to produce the original sequence devised for the normal left limb, then the movements exhibited by the transplanted limb in the various phases of locomotion should be expected to be the exact mirror images of the ones that the normal limb would perform at the same instant. This was actually found to be the case in salamanders (11 successful cases) in which the developed fore limbs had been interchanged. These limbs moved always in the reverse direction. In the progressing animal they strode backward instead of forward, and although this interfered seriously with the locomotion of the body, the central pattern has never been changed and adjusted to the new conditions.

Salamanders with interchanged right and left fore limbs, displaying reversed locomotion. PAUL WEISS (introduced by R. W. Gerard). Department of Zoology, The University of Chicago. (Demonstration.)

A study of the choline oxidizing enzyme. MARY SCOTT WELCH (by invitation) and LAURENCE IRVING. Department of Physiology, University of Toronto.

Welch, Irving and Best reported (1935) that when choline was added in vitro to liver slices the oxygen consumption was markedly increased. Trowell, later, reported similar results and suggested that as judged by the amount of extra oxygen consumed the choline was oxidized.

The increase in oxygen consumption is greater and can be more regularly reproduced when choline is added to liver brei rather than to tissue slices, and we have therefore used the brei for experiments to investigate the enzymatic process which is involved. That choline is oxidized by liver brei is shown definitely by the biological assay of the material remaining in the respirometer vessels. In glycerophosphate buffer (pH 7.4) choline consumes 1 atom of oxygen per molecule of choline.

A direct proportionality does not exist between the extra oxygen consumed and the amount of choline that is added. The extra oxygen consumed per mg. of added choline is maximum at 0.5 to 1.5 mgm. Higher concentrations cause a definite decrease in the oxygen consumption per milligram of choline. The same holds true when the concentration of enzyme is varied with the concentration of choline kept constant. The maximum effect is obtained when 0.3 to 0.5 cc. liver brei is used while, with higher concentrations there appears to be an inhibiting factor present in the liver which prevents the oxidation of the choline to so great an extent.

Brain, spleen, suprarenal gland, testis, smooth muscle, and striated muscle do not contain the oxidizing enzyme while kidney is approximately as active as liver in oxidizing choline.

The enzyme is almost completely inhibited by urethane and to the extent of about 60 to 70 per cent by NaCn. Choline increases the rate of reduction of methylene blue by liver. These results suggest that the enzyme is a dehydrogenase.

The effect of theelin and amniotin upon the BMR and the hot flashes of ovariectomized women. GRACE E. WERTENBERGER (by invitation), MARY E. COLLETT and JOSEPH T. SMITH (by invitation). Departments of Biology and Gynecology, Western Reserve University, Cleveland, Ohio.

The subjects were colored women who had undergone surgical removal of both ovaries, tubes and uterus, and were observed three to nine months after operation. We are indebted to Professor Bill and Doctor Weir of the Department of Obstetrics and Gynecology for patients and supplies. Case E was 25, case F 30, and case G 41.

In case E single doses of oil theelin (1000 I.U.) produced an alternate rise and fall of 6 per cent from the initial level. Intensive dosage (1 cc. daily for six days) produced a fall during medication of 10 per cent, followed by a rise of 7 per cent which lasted more than four weeks.

In case F single doses of oil theelin resulted in fluctuation of plus and minus 5 per cent from the initial level. Intensive dosage produced a fall during medication of 13 per cent, followed by a rise of 13 per cent above the initial level which lasted over four weeks. Amniotin capsules (1000 I.U.) were given orally, one capsule daily for twenty days to cases E and G. Case E showed a fluctuation of plus and minus 7 per cent from the initial level during medication, and a prolonged rise of plus 9 per cent in the following three weeks. Case G showed a fluctuation of plus and minus 6 per cent from the initial level during medication, and a prolonged rise of plus 12 per cent during the three weeks following.

Nervous symptoms and hot flashes are not subject to mensuration. Some *post hoc* changes occurred, even if not necessarily *propter hoc*. Numerous and severe hot flashes accompanied low BMR readings, especially sudden drops in rate. They were lighter and fewer when the rate was high. Interruption of a course of intensive treatment was often followed by an increase of symptoms. In cases G and E the relief from symptoms persisted longer after a course of oestrogenous hormones by mouth than after a series of injections.

Our results confirm those we reported last year on theelin by injection, and present the further point that orally administered amniotin is active.

The molecular weight of inulin and the mode of its renal excretion in Amphibia. B. B. Westfall and James P. Hendrix (introduced by A. N. Richards). Laboratory of Pharmacology, University of Pennsylvania.

The vapor pressure method of A. V. Hill as developed by Baldes has been applied to the determination of the molecular weight of inulin. The maximum value obtained in 4850: the average of five separate determinations regarded as most perfect technically is 4450. These values are similar to those obtained with other methods by Haworth and Irvine and their collaborators: higher than those obtained by numerous other workers.

Analyses of glomerular urine from frogs which had received inulin intravenously showed that this polysaccharide is completely filterable through the glomerular membrane. Perfusions of the frog's kidney gave no indication that inulin is secreted by the tubule.

A conductivity cell with a capacity of a few tenths of a cubic centimeter. H. L. White. Department of Physiology, Washington University School of Medicine, St. Louis. (Demonstration.)

The removable electrode is sealed into the male element of a ground glass joint, providing a means of obtaining reproducible interelectrode distance. The design permits a considerable range in cell constant and amount of material required; the practical minimum amount is about 0.2

cc. The cell demonstrated takes about 0.5 cc.; it has a resistance of about 680 ohms with normal human serum and readings are reproducible to 0.1 per cent.

The threshold stimulus of the knee jerk as an index to physical fitness. G. C. WICKWIRE (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

In the work reported in the literature, the extent of movement of the foot is used as an index to the knee jerk. In this investigation the threshold or minimal stimulus necessary to elicit the jerk was determined. The usual method was employed for evoking the knee kick, namely, by striking the patellar ligament with a hammer swung through a fixed arc. A weight carrier was attached to the hammer and the strength of the blow or stimulus was increased or decreased by adding or removing 10 gram weights.

It was found upon arising in the morning that a blow with a comparatively light hammer produced a kick but as the day wore on it was necessary to increase the weight of the hammer progressively by adding 10 gram weights to elicit a knee jerk and that by the time of retiring in the evening it was necessary almost to double the original weight of the hammer. Upon retiring there was an abrupt decrease in the weight of the hammer necessary to evoke the jerk, and this continued throughout the night until the next morning when a hammer as light as the one used on the preceding morning produced a kick.

If the day's work, either physical or mental, was very strenuous it required a much greater increase in the weight of the hammer to elicit a kick than when the day's work was light. It was also found that when the physically fit performed the same task as the physically unfit a much less increase in the weight of the hammer was required to produce a knee kick in the physically fit than in the unfit. After the performance of the work, only a short time was required before the original light weighted hammer would evoke a knee jerk in the physically fit whereas a much longer time was required in the physically unfit. Hence, it would seem that the threshold stimulus for the knee jerk may be used as a test for physical fitness.

Cardiac massage followed by countershock in revival of mammalian ventricles from fibrillation due to coronary occlusion. C. J. WIGGERS. Western Reserve University, School of Medicine.

To minimize the sacrifice of animals in studies upon the coronary circulation we have employed countershock applied through padded electrodes as described by Hooker, Kouwenhoven and Langworthy (Am. J. Physiol. **103:** 444, 1933). We found, as they did, that brief passage through a heart of a 60 cycle A.C. current having a minimal strength of 1 Amp. stops fibrillation and is followed spontaneously by strong coördinated contractions capable of restoring a normal blood pressure. This is only true however provided the coronary occlusion is removed and fibrillation has not lasted more than 2 or 3 minutes. If fibrillation has existed 5–8 minutes, restoration is not often successful even with the use of massage and epinephrine *subsequent to* stoppage of fibrillation. Since, in practice the two minutes for recovery expectancy are easily exceeded, any procedure that extends the time for hopeful recovery by only a few minutes becomes of practical importance.

By the slight but essential modification of massaging the heart *before* rather than *after* application of the countershock we were able in 40 out of 47 dogs to revive hearts in fibrillation for 5 to 7 minutes without use of drugs or chemicals, in many cases as often as 5 to 8 times.

The ventricles are manually compressed 40 x/min. each time forcing blood into the aorta, but without kneading the heart muscle as is implied by the term massage. Aided by the existing asphyxial vasoconstrictions, an aortic pressure of 60–85 mm. for feeding the coronary system can generally be maintained. Countershock applied 30–60 sec. after such artificial perfusion, is followed by vigorous beats, blood pressure frequently increasing far above the normal. The success of the procedure is explained by demonstrations of Tennant and Wiggers that effective contraction of the working ventricle cannot occur after complete ischemia of about one minute.

The method should prove of value in revival of exposed human hearts that fibrillate accidentally during course of cardiac operations.

Time relations of monophasic action potentials from the ventricle. HAROLD C. WIGGERS (introduced by C. J. Wiggers). Western Reserve University, School of Medicine.

In monophasic leads from an injury on the dog's left ventricle and a viable point on the right, the period of sustained negativity remains constant two or more minutes after occluding the *ramus descendens anterior*, but when localization of injured and viable points is reversed, it is reduced significantly. Obviously potential changes at uninjured regions are alone responsible for this reduction. Since during ischemia the amplitude of monophasic curves and relation of their ascent to R_{II} remained constant, the assumption was made that a greater positivity at the point of injury determines the initial rise.

Recent studies have shown that such constancy of the rise occurs only when the ascending limb and/or isopotential line display oscillations or notches; when these are absent the rise is somewhat delayed.

Triple leads were simultaneously recorded from A, injured area ↔ leg; B, uninjured area ↔ leg and C, injured ↔ uninjured areas. When curves from lead C possess oscillations, the initial rise often coincides with initial positivity of lead A,—but when they are absolutely smooth, the rise begins definitely later. This suggests that potential alterations at the injured region may be responsible for the initial rise of monophasic curves that are impure, but cannot explain those that display no superimposed waves either immediately preceding or during the rise. The corollary follows that conditions for leading from an injured area to the body are different than for leads between injured and uninjured spots on the heart.

By shifting the uninjured lead over the anterior epicardial surface, preliminary analyses indicate that the ventricular surface is nearly simultaneously negative. This supports the original discarded view of Erfmann and suggests that the unipolar methods used by Lewis and Rothschild and subsequently by C. J. Wiggers may not have been sufficiently exact to establish incidence of negativity over different regions. Perhaps some revision of conceptions regarding velocity and spread of impulses through the myocardium may be required.

The influence of the pylorus on the secretion of acid by the fundus. CHARLES M. WILHELMJ, F. T. O'BRIEN (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska.

A 2 per cent Liebig's Extract Test Meal containing phenol red was used in all experiments.

1. *Whole stomach pouches.* Four whole stomach pouches containing the pyloric portion were compared with five whole stomach pouches from which the pyloric portion had been removed. The rate of acid secretion was much lower in the pouches without the pyloric segment.

2. *Intact dogs.* Four dogs were used. In two the acid secretion was studied before and after making a gastroduodenostomy with large stoma in order to admit large amounts of duodenal secretions into the stomach.

Two dogs were studied before and after removal of the pyloric portion of the stomach and a Polya anastomosis to the duodenum.

When full allowance was made for the diluting and neutralizing effect of the duodenal secretions it was found that removal of the pyloric portion caused a definite lowering of the rate of acid secretion which was not seen after gastroduodenostomy in which large amounts of duodenal secretion entered the stomach.

The normal gastric secretory curve using a two per cent Liebig's extract test meal. CHARLES M. WILHELMJ, F. T. O'BRIEN (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska. (Read by title.)

When a 2 per cent Liebig's Extract Test Meal containing phenol red is used it is possible to determine the relative amounts of *acid* and *non acid* fluid entering the stomach during the secretory period.

A study of four whole stomachs isolated from the duodenum showed that the amount of non acid fluid secreted by the stomach is small in amount and usually decreases as the experiment progresses.

A study of 11 normal, intact dogs showed that there is usually an abrupt and marked increase in non acid fluid, just before the stomach empties, accompanied by the appearance of bile in the gastric contents.

A comparison of the secretory curves in isolated and intact stomachs shows that regurgitation of duodenal secretions is a normal event. Absorption of water from the stomach is often a factor in offsetting the effects of duodenal regurgitation.

Carotid sinus vasomotor reflexes. CLAUDE V. WINDER (introduced by Robert Gesell). Department of Physiology, University of Michigan. (Read by title.)

A continuous artificial circuit with continuous gaseous equilibration of the perfusing fluid has been used in a study of carotid sinus reflexes in the dog. Results emphasized that the vasomotor reflex arising from the carotid sinuses is a very definite and constant entity. For physiological, gradual changes in systemic arterial pressure near the normal level its correcting influence on that pressure is wonderfully sensitive and exact. Its *maximal* stimulation is capable of causing maximal, maintained, general vascular relaxation. Provided that the cardio-aortic nerves, normally

acting synergically with the sinuses in responding to a common arterial pressure change, are not allowed under experimental conditions to antagonize, complete removal of tonic sinus stimulation appears to completely unbridle vasomotor activity. Overshooting and falling back of blood-pressure responses to unnaturally abrupt changes in stimulus level at the sinus, studied both before and after "vagotomy," furnish interesting demonstrations of the known vicarious activity of the cardio-aortic nerves and are also amenable to correlation with characteristics of sinus receptor discharge observed by Bronk and Stella. The controversial inverted response to changes in level of sinus stimulation below 40 to 60 mm. Hg pressure in the dog, depends on a *pulsatile* stimulating pressure.

Compensatory adrenal hypertrophy in the rat. CHARLES A. WINTER (by invitation) and FREDERICK E. EMERY. Department of Physiology, University of Buffalo.

When one adrenal of the rat is removed, the compensatory hypertrophy which occurs in the remaining adrenal takes place quickly. Animals which have had one adrenal out for several months show no greater degree of hypertrophy than those which have been unilaterally adrenalectomized for a few days only. It has previously been shown that the adrenal enlargement which follows administration of beef anterior lobe extract or tissue to rats does not occur in thyroidectomized recipients, but that compensatory hypertrophy following removal of one adrenal does occur in thyroidectomized rats. We now have evidence that compensatory hypertrophy is indeed as great in thyroidectomized rats as in normals. This might be interpreted to mean that the pituitary has two adrenotropic factors, one needing the presence of the thyroid for its action, the other working with the thyroid either in or out, and just as effective with the thyroid removed.

Blood pressure tracings of small animals. R. A. WOODBURY (introduced by W. F. Hamilton). Department of Physiology and Pharmacology, University of Georgia, School of Medicine, Augusta, Georgia.

Intracardiac and intra-arterial blood pressure measurements have been recorded from canaries, sparrows, pigeons, mice, rats, frogs, turtles and fish by means of a hypodermic manometer. With this manometer one obtains accurate systolic and diastolic blood pressures and a true tracing of the contour of the pressure pulse even with a heart rate as rapid as 900 per minute without sacrificing the animal.

The hearts of these small animals were entered with a 26 G. needle, either by piercing the body wall or by exposing the heart where such procedure did not interfere with respiration. The frequency of the manometer system was adequate when equipped with a 26 G. needle.

In order to enter the arteries of the smaller animals glass cannulae were used. These were prepared with a very abrupt taper, the lumen at the tip being 0.05 mm. in diameter—one-fifth that of a 26 G. needle. Their small size made it possible to insert them directly into an exposed artery without cutting the wall. Their abrupt taper prevented bleeding around the cannulae and reduced the effective mass to a value lower than that of a similar manometer equipped with a 26 G. needle one-half inch in length. The frequency of the system was therefore entirely adequate and damping occurred almost immediately.

Warm blooded animals were found to have blood pressure values equal to or above those in man, while the cold blooded animals were found to have systolic readings from 35 to 60 mm. of mercury and a pulse pressure averaging 9 mm. of mercury. The heart rates of some small warm blooded animals were as rapid as 900 per minute.

A large part of the work was done at the Laboratory of the Department of Physiology, of the University of Wisconsin.

Cortical control of placing and hopping reactions in Macaca mulatta. CLINTON N. WOOLSEY (by invitation) and PHILIP BARD. Department of Physiology, Johns Hopkins University School of Medicine.

In accord with its greater motor versatility the monkey shows more highly developed placing and hopping reactions than the cat and one finds a correspondingly greater dependence of these reactions upon the cerebral cortex.

Following complete hemidecortication all these responses except the labyrinthine (which become greatly deficient) are totally and permanently abolished in the contralateral extremities while ipsilateral reactions remain entirely normal. Unilateral removals of *a*, all cortex anterior to the central fissure; *b*, areas 4 and 6 together, or *c*, the cortex corresponding to Brodmann's representation of area 4, cause enduring loss of all contralateral reactions except the visual and labyrinthine which become only deficient. Less extensive extirpations of area 4 produce permanent deficiencies but not complete losses. When a unilateral precentral ablation has produced a complete deficiency in placing reactions of the opposite limbs, placing (cross-placing) of the sound extremities occurs on appropriate tactile stimulation of the affected members. This indicates that the deficit consists in motor rather than sensory loss.

Placing the contralateral, and cross-placing of the ipsilateral extremities in response to tactile stimulation of the affected limbs are the only reactions completely and permanently abolished by parietal lobectomy or unilateral removal of all cortex except the frontal lobe. Following these ablations the hopping reactions (proprioceptive) are seriously affected but subsequently become normal.

Unilateral removals of all cortex anterior to Brodmann's area 6, or total ablation of occipital and temporal lobes produce but slight transient effects. When both ablations are carried out in one hemisphere the sensorimotor remnant manages the reactions normally.

Our results demonstrate the high degree of localization of the cortical control of these postural reactions in the monkey and yield evidence that the cortical representation of tactile sensibility is chiefly in the parietal lobe while that of proprioceptive sensibility lies mainly ahead of the central fissure.

Motion picture of placing and hopping reactions in Macaca mulatta and the effects upon them of various cortical ablations. CLINTON N. WOOLSEY (by invitation) and PHILIP BARD. Department of Physiology, Johns Hopkins University School of Medicine. (Demonstration.)

The distribution of adrenergic vasodilators in the rat. LELAND C. WYMAN and CAROLINE TUM SUDEN (by invitation). Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, Massachusetts Memorial Hospitals.

In the cat sympathetic (adrenergic) vasodilators are distributed mainly to the periphery, chiefly to skeletal muscles, while the splanchnic vasomotor innervation is primarily constrictor (Hartman, 1915; Rosenblueth and Cannon, 1933). This has been deduced from the study of vasodilator responses to small amounts of adrenalin, or of adrenergic dilator responses revealed by ergotoxine in animals with peripheral or splanchnic circulation reduced by ligation. That the rat possesses adrenergic vasodilators, contrary to previous opinion concerning rodents, may be demonstrated after inactivation of the suprarenal medulla or after sufficient doses of ergotamine tartrate. Direct observation of vessels has shown that the splanchnic circulation may be involved in the dilator responses to small doses of adrenalin.

Following reduction of the splanchnic circulation by ligation of coeliac and superior mesenteric arteries, the pressor responses to small amounts of adrenalin (0.05 or 0.1 cc. of 1:1,000,000) were, in normal rats, reduced about one half; while those to larger doses (0.05 or 0.1 cc. of 1:100,000) were slightly reduced or unchanged. The depressor responses to small doses in rats with the suprarenal medulla eliminated by ligation or by suprarenalectomy were somewhat decreased. The depressor responses to larger doses, revealed in normal rats after treatment with ergotamine, were unchanged.

Following reduction of the peripheral circulation by ligation of subclavian arteries and abdominal aorta above the iliacs, the pressor responses to small amounts of adrenalin in normal rats were consistently increased, while those to larger doses were unchanged or slightly increased. The depressor responses to small doses in rats with the suprarenal medulla eliminated were much decreased. The depressor responses to larger doses revealed by ergotamine in normal rats were unchanged.

These findings are consistent with the conclusions that in the rat, as in the cat, adrenergic vasoconstrictors are more abundantly distributed to the splanchnic area as compared with the peripheral area; but that the rat, unlike the cat, has a significant distribution of adrenergic vasodilators to both regions, perhaps even an almost equal distribution. Such interspecific differences in vascular physiology are of significance when general conclusions concerning the physiology of mammals are to be drawn.

The adrenals and gonads of rats, following thyroidectomy, considered in relation to pituitary histology. ISOLDE T. ZECKWER. University of Pennsylvania Medical School, Philadelphia.

Rats, thyroidectomized between 23 and 52 days of age, were killed from 31 to 250 days after operation. Organs were weighed and studied microscopically. Kidney weights gave a better index of dwarfing than did body weights, because thickened subcutaneous tissues in cretins masked the dwarfing. In male rats absolute weights of adrenals and testes though usually reduced were close in value to those of litter-mate controls, although the kidneys of cretins might be only .4 of the weight of controls. In females the absolute weights of adrenals and ovaries were reduced to about the same degree as were the kidney weights.

The dissociation of growth of adrenals and gonads from the growth of the kidneys, in males, and, on the contrary, the correlation of retarded growth of adrenals and gonads with the degree of retardation in growth of kidneys, in females, was not reflected by any obvious sex difference in the

histology of the cretins' pituitaries. Perhaps differential counts in progress now, may show a sex difference. In both sexes cretin pituitaries showed early diminution in number of acidophiles even to the point of complete disappearance, and a great increase in basophilic cells distended with hyaline material which apparently represents stored secretion. In late periods acidophiles were present but decreased in number below normal. The early skeletal and visceral dwarfing of cretins is attributed to loss of acidophiles. The maintenance of adrenal weight in the male when the pituitary lacks acidophiles, probably excludes the acidophils as the producer of adrenotropic hormone. In implantation experiments in progress it is found that cretin pituitaries contain a very large amount of thyrotropic hormone. Acidophiles can, therefore, probably be ruled out as producers of thyrotropic hormone, which is probably produced by the basophilic cells containing the stored hyaline. These "thyroidectomy cells" differ histologically from "castration cells" although they have basophilic cytoplasm. Probably cells with basophilic reaction produce the adrenotropic hormone as well as thyrotropic and gonadotropic hormones.

Effects of roentgen rays upon the osmotic behavior of the erythrocyte. R. E. Zirkle and C. J. Garrahan (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania.

When the beef erythrocyte is treated with roentgen rays the permeability to various substances, as measured by the method of osmotic hemolysis, is altered by widely varying amounts. A dose of 30000 roentgens has no appreciable effect upon the rate of entrance of glycerol but accelerates the entrance of a related compound, ethylene glycol, by 15 per cent. After the same dose, water enters over 1.5 times as fast. For this substance, easily measurable changes in the rate of entrance are brought about by 5000 roentgens. When the rate of hemolysis is plotted against dosage, the resulting curve rises rapidly up to 10,000 roentgens but thence has a tendency to flatten.

Irradiated erythrocytes of the pig and the rat resemble those of the beef in showing no change in permeability to glycerol, even though the normal time of hemolysis for the pig is 50 times that for the rat.

For the pig erythrocyte the rate of entrance of water is increased to approximately the same extent as the beef but no measurable change occurs in the case of the rat.

THE POST-EXERCISE SUPPRESSION OF KIDNEY FUNCTION IN MAN

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Violent exercise is associated with a modification of kidney function characterized by diminution of excretory rate, increase in urinary cH, and the appearance of abnormal constituents in the urine. MacKeith and his co-workers (1923) demonstrated anuria or oliguria as a constant finding during running in man when excretory rate had been augmented by a diuretic. They suggested that the suppression of kidney function was the result of a decreased blood supply produced by an overflow of constrictor impulses to the renal vessels. This hypothesis was subsequently directly tested on dogs (Klisiecki et al., 1933a) by denervating the kidney and measuring exercise changes in rate of urine flow. The renal nerves played no part in the inhibition of the response of the kidneys by exercise. Although in the experiments on man diuresis was promptly resumed with the cessation of work, the suppression of excretory function markedly outlasted the period of activity in the dog. We had incidentally noted that oliguria also persisted in the human in spite of the ingestion of diuretic doses of water administered in an effort to facilitate post-exercise urine analysis by increasing the volume of the samples voided. The object of this study was to investigate further this phenomenon in man.

METHODS. The subjects were healthy young adult women students majoring in physical education and accustomed to the performance of vigorous exercise. They were taught to void at short intervals, completely emptying the bladder. The excretory response of the trained fasting subject was highly repeatable when special precautions were taken to procure normal water balance prior to the production of diuresis, and experimental conditions were kept scrupulously constant. After the determination of basal excretory rate moderate diuresis was induced either by the administration of 500 cc. of water, or by drinking 100 cc. every 15 minutes until 1000 cc. had been taken, since single large doses are not well tolerated and interfere with the performance of severe exercise. Observations were continued until the excretory rate subsided to approximately normal. Most of the evidence was obtained after measured work performed on

the electro-dynamic brake bicycle ergometer; a few observations were made after participation in natural sport activities, walking, and rope jumping.

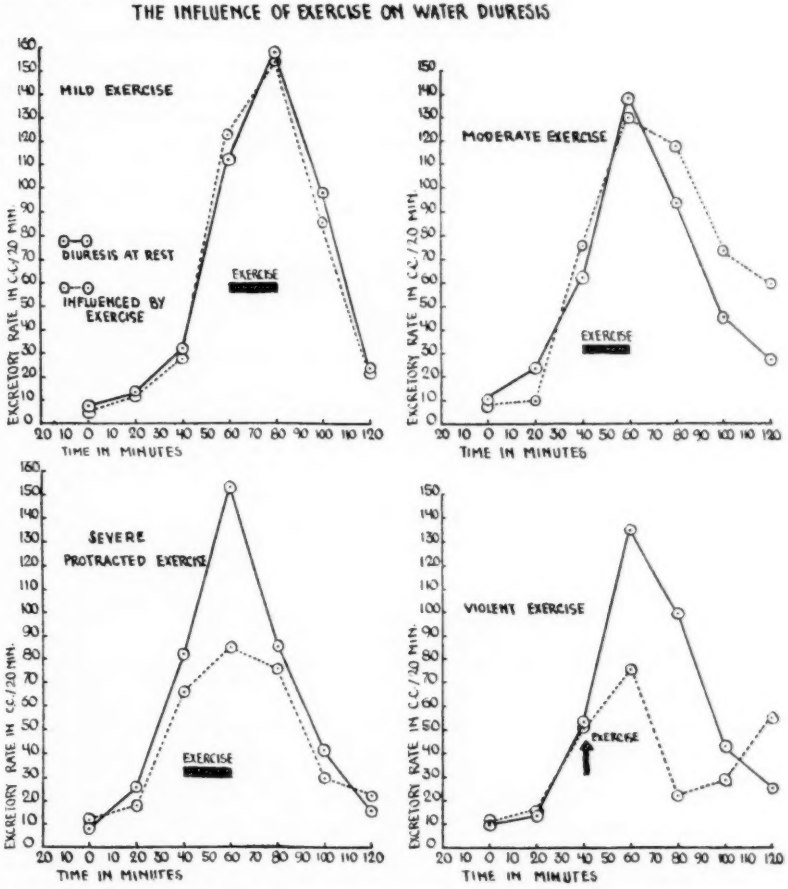

Fig. 1. The influence of various types of exercise on the diuresis produced by 500 cc. of water taken in a single dose. Exercise was introduced after absorption was approximately complete and when the maximum load of absorbed but unexcreted water was present in the tissues.

RESULTS. *1. Normal diuresis.* The diuretic response at rest shows certain constant features. The administration of 500 cc. of water was attended by a delay of 20 minutes before excretory rate appreciably increased. Maximal output was attained 60 to 80 minutes after the inges-

tion of water. At peak diuresis the rate of excretion was approximately 15 times normal. The total fluid recovered before urine production returned to normal varied from 60 to 100 per cent, depending upon the adequacy of the previous hydration. The administration of 1000 cc. of water in small divided doses was accompanied by a more prolonged delay before the onset of diuresis. An output approximately equivalent to intake was maintained for 60 minutes, thus giving a rounded instead of a sharply peaked diuresis curve. The two types of response are illustrated in the normals presented for comparison in figures 1 and 2.

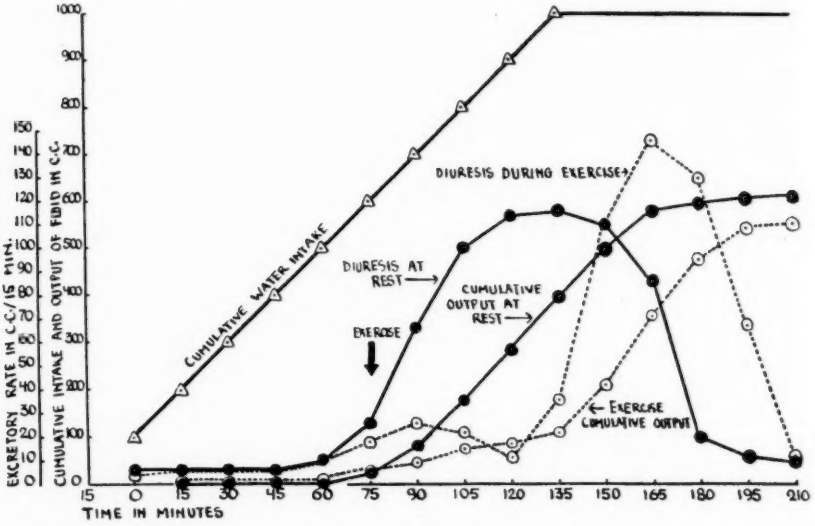

Fig. 2. The influence of brief violent exercise on the diuresis produced by 1000 cc. of water taken in divided doses, 100 cc./15 min.

2. The influence of various types of exercise upon water diuresis. Four types of muscular activity were studied. Mild exercise consisted of walking out of doors. Exercise of moderate severity was performed on the stationary bicycle and was carried on in the steady state. Rotational bouts of exhaustive work on the bicycle ergometer, hand ergometer, treadmill and rowing machine comprised the severe exercise. It was accompanied by sweating and dyspnea. These three types of exercise were of 20 minutes' duration, occupying the interval immediately preceding the expected greatest increase in excretory rate. Violent exercise lasted for 3 minutes and was performed on the bicycle, working at the rate of 1031 kgm.m./min. at 88 R.P.M. Sixty-six observations were made on 23 subjects. Average results are illustrated in figure 1. Twenty-eight ob-

servations on 10 subjects were made after divided doses of water. Exercise was introduced in these experiments *immediately after* the onset of diuresis. A typical experiment is illustrated in figure 2.

Mild exercise had no influence on water diuresis. Excretory rate during the course of moderately severe work was also unaffected, but post-exercise urine formation was stimulated. Mean recovery reached 101.2 per cent as compared with 80.8 per cent at rest. Severe protracted exercise inhibited diuresis during the period of activity. Excretory rate closely approximated normal after the cessation of work. Only 59.4 per cent of the extra water ingested was recovered. The discrepancy between intake and output may be accounted for by extra-renal water loss. Three minutes of violent exercise suppressed diuresis for 60 minutes, after

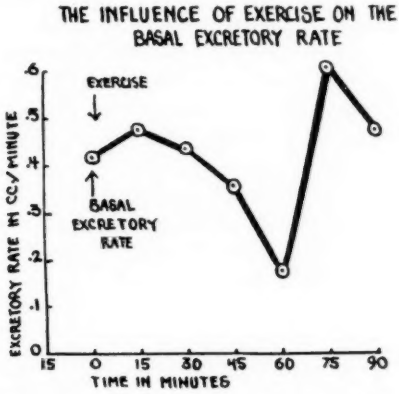

Fig. 3. A composite of 5 observations on 1 subject showing the influence of brief violent exercise on the rate of urine formation of the kidney unstimulated by excess water.

which excretory rate again rose. Maximal inhibition was not attained until 40 minutes after the period of exercise. Subsequent experimental work demonstrated that this post-exercise suppression is a constant phenomenon associated with the recovery from all types of exercise during which a large oxygen debt is incurred. It has been observed following rope jumping, standing running and field hockey. It occurs whether exercise precedes or follows the ingestion of water, and whether fluids are taken in single or divided doses. Oliguria is always profound, the rate of urine formation subsiding to a level which approximates basal excretion, irrespective of the height to which function has been stimulated at the moment exercise is introduced. A lag in the attainment of the greatest suppression of function is also a constant finding. If a brief exercise of speed is carried to the point of exhaustion, the smallest post-exercise vol-

ume is invariably the sample collected 45 minutes after the onset of the 3 minute period of work. Diuresis is then resumed, and the excess water which has been temporarily held back, is promptly eliminated. Peak diuresis during the delayed response not infrequently exceeds that attained at rest as illustrated in figure 2.

3. The influence of violent exercise upon basal excretion rate. The kidney unstimulated by excess water is also affected by exercise. Sixteen observations were made on 4 subjects. In its essential features the response was identical to that evident during diuresis. Excretion rate was at first stimulated, then suppressed. After a long lag, urine formation fell to its lowest level. As illustrated in figure 3, at times it then abruptly increased, being temporarily augmented to a rate above normal, as though the fluid retained immediately post-exercise was suddenly made available for excretion.

COMMENT. That the suppression of kidney function post-exercise is not due to failure in absorption is evident because typical inhibition occurred when exercise was introduced after diuresis had been well established. The experiments of Heller and Smirk (1932) on rabbits, rats and guinea pigs, of Klisiecki et al. (1933b) on dogs and of Smirk (1933) on man demonstrated that the alimentary absorption of water is well advanced before any appreciable increase in the rate of urine formation takes place and is complete 15 to 20 minutes before the attainment of peak diuresis. In the experiments illustrated in figure 1, exercise was introduced 20 minutes before peak diuresis. The vain attempt of the kidney to produce urine after certain types of exercise cannot therefore be attributed to stagnation of fluid in the gut with diminution of the tissue water load.

Contracting muscle has an augmented avidity for water, presumably as a result of the increased concentration of osmotically active components. This has been observed by Barcroft and Kato on the dog (1916), and by Black, Cogan and Towers on the frog (1915). Gregersen (1936) has found a decrease in plasma volume as a result of exercise in the human. Dill, Talbott and Edwards (1930) have reported hemoconcentration in exercise. Margaria (1930) observed a great increase in the osmotic pressure of the blood following short violent muscular work by man. The ingestion of water in doses 2 to 4 times as large as ours produced relatively less change in the opposite direction. Violent exercise depletes the blood of water. Further, cardio-vascular adjustments are slow after brief violent muscular exertion. A prolonged negative phase ensues, during which the pulse pressure drops to levels below normal (Hellebrandt, 1932). There results thus, a disturbance in the balance between hydrostatic pressure and colloid osmotic pressure, the exercise deviations in each tending to limit the volume of glomerular filtrate. Peters (1935)

has recently placed emphasis on the dependence of urine volume on blood volume and the prime importance of hydrostatic and colloid osmotic pressures in the shifts of body fluids. It is suggested that herein may reside a plausible hypothesis, accounting for the prolonged oliguria which follows upon brief violent exercise.

SUMMARY AND CONCLUSIONS

Mild exercise has no influence on water diuresis. Post-exercise urine formation is stimulated by moderate activity. More rigorous exertion associated with breathlessness and sweating inhibits diuresis during the period of work. Brief violent exercise such as is accompanied by lactic acid acidosis and a large oxygen debt suppresses kidney function for 45 to 60 minutes. There is a lag of 45 minutes before the greatest excretory diminution occurs. When diuresis is resumed the rate of urine formation may exceed that attained at rest. The prolonged arrest of diuresis after brief violent exercise cannot be attributed to failure in absorption. The hypothesis suggested is that kidney function is suppressed after violent exercise because: 1, blood is depleted of water by virtue of the increase in concentration of osmotically active components in the muscles; 2, a prolonged negative phase in blood pressure ensues; and 3, a resultant disturbance in the balance of osmotic and hydrostatic pressures reduces the volume of glomerular filtrate until the cardiovascular and physicochemical disturbances of exercise subside, 45 to 60 minutes after the cessation of muscular work.

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COMPARISON OF CHANGES IN THE pH OF ARTERIAL BLOOD AND SALIVA DURING VARIATIONS OF PULMONARY VENTILATION[1, 2, 3]

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The present investigation was undertaken in the hope that further light might be thrown upon the acid-base changes within tissues under conditions which affect the respiratory mechanism and the reaction of arterial blood. Eddy (1930a, b) found when the submaxillary gland of the dog was made to secrete at a constant rate by continuous injection of pilocarpine, that intravenous administration of sodium bicarbonate, sodium carbonate, or sodium hydroxide always decreased the rate of secretion. Evidence indicated that the decrease in secretion caused by these substances was due primarily to changes in acid-base equilibrium of the blood and tissues, in which the hydrogen-ion concentration of the interior of the cell may be a major factor. Administration of carbon dioxide or intravenous injection of hydrochloric or lactic acid produced an increase in secretory rate which also was attributed to changes in the acid base equilibrium of the tissues and blood. Should the saliva reflect hydrogen-ion changes occurring within the gland, it was thought that some information concerning the acid-base changes within the cells of the gland might be obtained by studying the changes in the pH of saliva brought about by experimental variations in the reaction of the blood. In this paper data are presented to show the effect of the administration of low oxygen and sodium cyanide on the pH of saliva as compared with that of arterial blood.

METHODS. Dogs varying in weight from 9 to 25 kgm. were used for the experiments. All animals were anesthetized by the injection of morphine and urethane. Mean blood pressure, respiratory rate, pulmonary ventilation and oxygen consumption were recorded in the usual way.

The right femoral vein was connected to a pump which delivered pilocarpine chloride or nitrate, 1:80,000 in 0.9 per cent sodium chloride

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[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

[3] This study was supported in part by a grant from the Rockefeller Foundation.

solution, at such a rate as to give a saliva secretion of 0.2 to 0.5 cc. per minute. To initiate secretion at the beginning of the experiment, 50 cc. of the pilocarpine solution were injected rapidly by means of a burette attached to the femoral vein. Since the animals varied in size as well as in sensitivity to pilocarpine the rate of injection was adjusted for each animal used. A Gibbs drop recorder delivering 25 drops per cubic centimeter was used to record the flow of saliva from the right submaxillary duct.

In a number of experiments 1 cc. samples of saliva for pH determinations were collected under oil at intervals of five minutes except during those procedures which prohibited such rapid collections due to decrease in the rate of secretion. These samples were stored in a small ice box until the end of the experiment. In some cases, however, the pH determinations were made immediately after collection of the sample. Storing the samples for periods of 6 to 8 hours apparently had no effect upon the pH. In the remaining experiments, pH was recorded continuously by mounting a glass electrode in the flowing saliva.

Blood samples were withdrawn from the left femoral artery and the determinations made immediately, or the samples were chilled and the determinations made at the end of the experiment. Heparin was used to prevent coagulation.

All pH determinations were made at body temperature with a glass electrode of the membrane type and an electrode thermoregulator as devised by Stadie, O'Brien and Laug (1931). A vacuum tube amplifier arrangement described by Hill (1931) was used to measure the potentials in the sample determinations, while the potentiometer set-up of Stadie et al. (1931) was used for the readings during continuous recording. The latter arrangement has the advantage of a very stable zero, which is necessary for continuous measurements. The E.M.F. readings were recorded continuously on smoked paper by means of the arrangement devised by Gesell and Hertzman (1926).

One-cubic centimeter samples were amply sufficient for duplicate determinations. Readings on the same sample of whole blood check very closely (less than 0.01 pH deviation); but readings on saliva are not so consistent for variations as great as 0.03 pH have been noted between duplicate determinations.

RESULTS AND DISCUSSION. In fourteen animals the pH variations of saliva and blood were followed during resting conditions. In the saliva the initial reaction and the subsequent changes were variable but the curve of pH plotted against time did not show abrupt variations over a period of two to three hours. The initial reaction from animal to animal varied between 7.11 and 7.65 with an average of 7.27 pH. In 8 cases there was an increase during a period of 2 hours of 0.05 to 0.40 pH. Three

animals showed a decrease of 0.15 to 0.22 pH while the remaining three showed first an increase and then a decrease in pH.

The initial reaction of the blood varied between 7.05 and 7.24 with an average of 7.13 pH. In 6 animals there was a subsequent increase of 0.01 to 0.07 pH while in the remainder there was a decrease of 0.01 to 0.18 pH. The variations in the initial pH of blood are doubtless associated with varying degrees of anesthesia. When in the course of an experiment it became necessary to administer urethane, this procedure was accompanied by a decrease in the blood pH. During these experiments pulmonary ventilation and mean blood pressure remained constant or varied

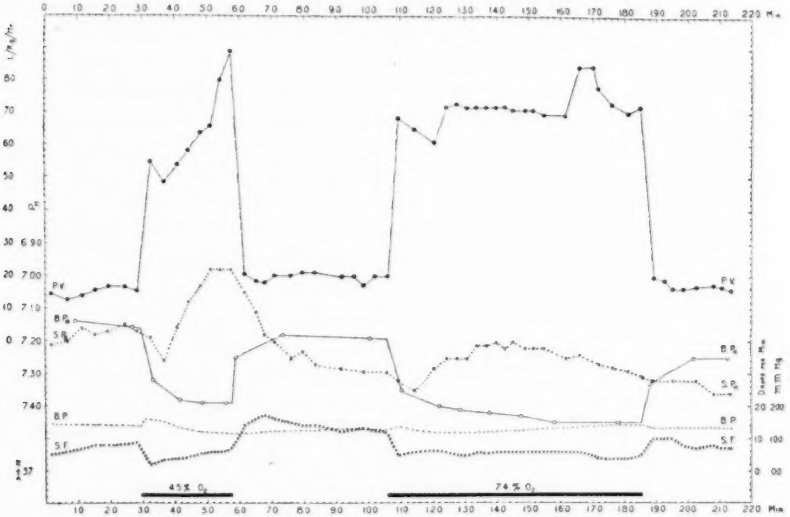

Fig. 1. Administration of low oxygen. $B.P_H$, blood pH; *P.V.*, pulmonary ventilation in liters per kilogram per hour; $S.P_H$, saliva pH; *B.P.*, blood pressure; *S.F.*, saliva flow in drops per minute, 25 drops equal 1 cc.

but little. It is to be noted that the changes in the saliva pH were invariably greater than those in the blood.

Using the quinhydrone electrode McClanahan and Amberson (1935) found from pH determinations of saliva and of arterial and venous plasma of the cat that the results are not always consistent, but that on the whole they indicate that salivary pH varies inversely as the pH of the arterial blood supplying the gland. The majority of our results do not show any inverse relationship between salivary pH and arterial pH in the anesthetized dog.

The first part of figure 1, in which the effects of the tracheal administration of 4.5 per cent oxygen in nitrogen are shown, illustrates the typical

results obtained by giving a dog an atmosphere containing less than 9 per cent oxygen. Inspection of this figure shows that arterial blood pH and pulmonary ventilation are increased throughout the whole period of administration; blood pressure increases at first then gradually decreases; saliva flow decreases at first then gradually increases; and saliva pH increases at first then shows a very marked decrease. On returning the animal to room air, pulmonary ventilation and blood pH show a rapid recovery; blood pressure recovers gradually; saliva flow increases rapidly above the preadministration rate and then declines to a level which is still above the initial rate; and saliva pH increases rapidly to a value greater than the preadministration level. However, in 18 out of 43 administrations of less than 9 per cent oxygen the initial increase in the saliva pH was absent. Still another type of variation in the saliva pH is illustrated by the second part of figure 1 which is a record of prolonged administration of 7.4 per cent oxygen. After the usual decrease, the saliva exhibits an increase in pH during the latter half of the procedure instead of the commonly continued decrease. This deviation occurred during 6 experiments in which the administrations were prolonged. In 25 experiments the administrations were prolonged until the blood pH decreased toward or below the preadministration values. In 22 of these the saliva pH decreased also, but in 3 the saliva pH showed an increase. In 21 of the same experiments the saliva flow diminished along with the decrease in blood pH.

In 6 experiments administration of 9 to 11 per cent oxygen for periods of 30 to 60 minutes produced only a decrease in both blood and saliva pH.

Only at the beginning of a low oxygen administration does the saliva pH seem to be affected in the same direction as the blood. As far as the blood pH is concerned the blowing off of carbon dioxide can account for the increased pH and it is likely that the diminution of CO_2 in the blood in turn produces the increased pH of the saliva by hastening the removal of CO_2 from the gland. This is attested by the results obtained upon administering 9 to 11 per cent oxygen. Such administrations lasting from thirty to sixty minutes produce an increase in pH of both blood and saliva but the increase in saliva pH is less than that of blood. Furthermore, hyperventilation with room air by means of a respiration pump also produces an increase in both blood and saliva pH with blood again showing the greater change.

The decrease in saliva pH following the initial increase during low oxygen administration is attributed rather to oxidative disturbances produced within the gland than to pH changes in the blood. The lower the percentage of oxygen given to the animal the sooner did the saliva show a decrease in pH. In some instances it was possible to produce the decreased pH in saliva by prolonged administration of 9 per cent oxygen.

Himwich and Adams (1929, 1930) found a marked increase in lactic acid formation during incubation of the submaxillary gland; Bergonzi and Bolcato (1931) found that phosphoric and lactic acids are liberated into the blood by the stimulated gland; and Northup (1935) showed that lactic acid increases while glycogen and creatine phosphate decrease when the gland is stimulated to exhaustion. Since Northup has shown that the submaxillary gland obtains energy for its secretory mechanisms from the same sources as does striated muscle in contraction, it is to be expected that similar changes may occur in each during anoxemia. The pouring out of lactic acid and phosphoric acid into the saliva could account for the decreased salivary pH during low oxygen administration despite the blowing off of carbon dioxide.

It is difficult to account for the fact that saliva pH increases in some cases with prolonged administration of low oxygen (fig. 1). In a previous paper from this laboratory (Gesell et al., 1932) calculations based on direct observation of the lactic acid increase and carbon dioxide loss indicated that muscle sometimes turned alkaline and sometimes acid during administration of low oxygen. If the saliva does reflect hydrogen ion changes occurring within the cells then we have evidence here which shows that the salivary cells too sometimes may turn alkaline and sometimes acid during the administration of low oxygen.

The improvement of oxidations on returning the animal to room air could account for the increase in pH of the saliva by the rapid removal of the acid metabolites and the restoration of the normal activity of the cells. It is to be noted that the saliva shows no marked change in pH on returning the animal to room air when the saliva showed an alkaline change during the latter part of the administration (fig. 1).

An initial decrease in rate of secretion was obtained when the oxygen percentage of the inspired air was decreased. This diminution in secretory rate was greater the lower the tension of oxygen. In most cases the secretion rate began to increase while the inhalation of low oxygen continued. In some this increase brought the secretion rate above that before the low oxygen was administered. However, if the administrations were prolonged a second decrease would occur, and if extended long enough to produce respiratory failure secretion would be stopped. When the animals were breathing room air again, the secretion rate showed a marked increase which then declined to a level which was usually above the preadministration level. Similar results have been reported by Eddy (1929).

With the initial decrease in flow the salivary pH may increase or remain unchanged. At least two factors are involved in the initial decrease in flow, a decrease of carbon dioxide, and an impairment of oxidations within the cells. The first factor would tend to produce an increase in

salivary pH while the second would tend to produce a decrease in pH. At the beginning of the procedure these two might balance as far as the reaction of the saliva is concerned. With the continued administration of low oxygen acids are formed which increase the secretory rate as well as diminish the salivary pH. In all administrations below 8 per cent in which the initial decrease in rate was followed by an increase in rate, the increase in rate was followed by a decrease in salivary pH (fig. 1).

The intravenous injection of M/200 solution of sodium cyanide produced salivary and blood pH changes similar to those obtained by the administration of low oxygen. Figure 2 shows the results of one of these experiments. The saliva shows an increase in pH following a similar but greater change in the blood. The increase in salivary pH is accompanied by a decrease in secretory rate. With respiratory recovery blood pH decreases to the preinjection level; saliva pH decreases beyond the preinjection level; and secretory rate increases above the preinjection flow, then decreases to the preadministration flow.

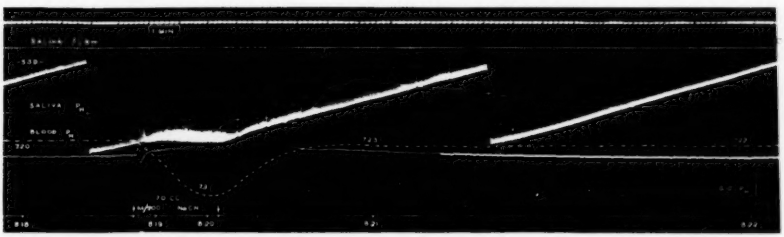

Fig. 2. Intravenous injection of sodium cyanide

The more intense the respiratory stimulation the more marked are the initial changes in blood pH, saliva pH and saliva flow. These changes are attributed to the removal of carbon dioxide by the increased ventilation. This conception is in line with the findings of Gesell and Hertzman (1928) on cerebrospinal fluid and of Gesell (1929) on lymph after cyanide injection.

Some of the acid metabolites produced within the gland during the anaerobic activity following cyanide injection may be excreted in the saliva and thus produce the decreased salivary pH. Ferrari and Hober (1933) found that in the isolated submaxillary gland of a cat a marked increase of lactic and phosphoric acids in the perfusate could be obtained by poisoning with KCN. While no data are available relative to the salivary content of these acids during cyanide poisoning the results presented here indicate that some acid substances are present. It is likely then that the accumulation of these substances in the gland accelerates the secretory rate despite the impairment of oxidations just as in the case

of low oxygen administration. If enough cyanide were given to produce respiratory failure for a time the secretory rate would increase for a short period and then stop. The saliva secreted during this period showed a marked decrease in pH. Blood also showed a marked decrease in pH but less than that of saliva, and somewhat later. With recovery of respiration secretory rate, saliva pH and blood pH would recover in that order.

Apparently then the saliva pH varies with that of the blood in these studies only during the early stages of low oxygen and cyanide administration. These changes are attributed to diminution of carbon dioxide as a result of the hyperventilation.

SUMMARY AND CONCLUSIONS

pH determinations were made by means of the glass electrode on blood samples taken from the femoral artery and on saliva samples taken from the submaxillary gland of anesthetized dogs. Continuous pH measurements also were made by mounting a glass electrode in the flowing saliva elicited by a continuous injection of pilocarpine.

A comparison of the pH values of saliva and arterial samples over periods of two hours or longer showed that while the arterial pH remained quite constant saliva pH showed large variations of which the majority were toward the alkaline side.

Lowering the oxygen tension of the inspired air caused an increase in the pH of arterial blood with a decrease toward or below the resting level if the administration were prolonged. The resting level was quickly reached upon returning the animal to room air. The pH of saliva increased at the beginning of the administration and then decreased below the pre-administration level in a majority of experiments. The initial increase was sometimes absent. If the administration were prolonged, a second increase might occur and in most cases a marked increase took place upon returning the animal to room air. The increase during recovery was usually above the pre-administration level and the pH frequently remained at this new level.

Injection of sodium cyanide caused an increase in both blood and saliva pH during the intense respiratory stimulation. If cessation of respiration occurred for a time both blood and saliva pH decreased but returned to pre-administration values with recovery of respiration, saliva showing a more rapid recovery than that of blood. If failure of respiration did not occur the blood pH returned to resting values but saliva pH showed a decrease accompanying the increased secretory rate and then increased to or above resting values with the return of the secretory rate to the preadministration value.

The initial alkaline change in the saliva with low oxygen administration or with cyanide injection is attributed to a decrease in carbon dioxide

produced by hyperventilation. The subsequent changes in the saliva pH are attributed more to disturbances of the cellular metabolism produced by the impairment of oxidations and the recovery of these processes than to the pH of arterial blood.

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THE DISTRIBUTION OF ADRENERGIC VASODILATORS IN THE RAT

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Sympathetic vasodilators, open to stimulation by adrenalin (adrenergic), have been studied largely in the cat and in the dog where they have been shown to be distributed for the most part to the peripheral circulation, and there chiefly to the skeletal muscles. The splanchnic vasomotor innervation is said to be primarily vasoconstrictor. This has been deduced from the study of vasodilator responses to small amounts of adrenalin, or of adrenergic dilator responses revealed by ergotoxine in animals with the peripheral or the splanchnic circulation reduced by ligation (Hartman, 1915; Hoskins, Gunning and Berry, 1916; Gruber, 1918; Cannon and Rosenblueth, 1933; Rosenblueth and B. Cannon, 1933).

That the rat possesses adrenergic vasodilators, contrary to previous opinion concerning rodents in general, has been demonstrated. Vasodepression in response to small amounts of adrenalin occurs after inactivation of the suprarenal medulla by ligation or excision of the glands (Wyman and tum Suden, 1932), and vasodepression in response to any effective amount of adrenalin occurs after treatment with sufficient ergotamine tartrate (Wyman and tum Suden, 1935). Direct microscopic observation of blood vessels showed that during the depressor responses to small amounts of adrenalin, following inactivation of the medulla, vasoconstriction occurred in the ear but that brief constriction followed by prolonged dilatation occurred in the vessels of the intestines and mesentery. This observation brought up the possibility of a difference between the manner of distribution of sympathetic vasodilators in the rat and that generally accepted for the cat and the dog. Herein is presented further evidence for this possibility.

METHODS. Carotid blood pressures were recorded by the mercury manometer in adult rats, 250 to 300 grams body weight, under urethane anesthesia. Intravenous injections were given in the femoral veins. The use of adrenalin chloride (Parke, Davis & Co.) and ergotamine tartrate (1 mgm. per cc.) solutions,[1] was controlled by water or saline injections.

[1] The ergotamine tartrate ("Gynergen II") was very kindly supplied by the Sandoz Chemical Works, Inc., New York.

Reduction of the splanchnic circulation was effected by ligation or clamping of the coeliac and superior mesenteric arteries. The inferior mesenteric artery in the rat is very small, and control injections of methylene blue or india ink showed that the bulk of the splanchnic circulation was excluded by the procedure described. To reduce the peripheral circulation the subclavian arteries were ligated close to their source and the abdominal aorta was clamped just above the ilio-lumbar arteries, while one carotid had already been excluded by cannulation.

RESULTS. *The effect of circulatory reduction on vasomotor responses to adrenalin in normal rats.* Upon ligation of the coeliac and superior mesenteric arteries there was a rise in the blood pressure level averaging about ten per cent of the pre-existing level (13 expts.). Following such reduction of the splanchnic circulation the heights of the small pressor responses to small amounts of adrenalin (0.05 or 0.1 cc. of 1:1,000,000) were reduced about one half, an average of 52 per cent in ten experiments. Pressor responses to larger amounts of adrenalin (0.05 or 0.1 cc. of 1:100,000) were not significantly changed or slightly reduced (from 20 to 30 per cent).

Upon ligation of the subclavian arteries and the lower abdominal aorta the blood pressure level was slightly increased in some experiments (from 3 to 8 per cent) although any increase was usually minimized either by rapid compensation or by a tendency to a fall in level due to the necessary operative manipulation which occasionally resulted in a somewhat lowered level. Following such reduction of the peripheral circulation the pressor responses to small amounts of adrenalin were consistently increased—an average of 43 per cent in five experiments. Pressor responses to the larger amounts of adrenalin were unchanged or somewhat increased.

That the changes in the pressor responses were not occasioned by the changes in blood pressure levels concomitant with either type of circulatory reduction was shown by the consistent appearance of the same modifications in cases where the blood pressure levels were not so altered, in cases where the level was altered in the opposite direction, and by the persistence of the modifications in experiments where the blood pressure later regained or passed its former level.

The effect of circulatory reduction on depressor responses to adrenalin. Immediately following removal or ligation of both suprarenal glands, small amounts of adrenalin (0.05 or 0.1 cc. of 1:1,000,000) produced depressor responses. Following reduction of the splanchnic circulation, these depressor responses were somewhat decreased—an average of 35 per cent in eight experiments. After reduction of the peripheral circulation they were much decreased, an average of 60 per cent in four experiments. In one case, where the response was of the pressor-depressor type before ligation of the peripheral arteries, the depressor component after ligation was decreased and the pressor component was increased.

The effect of circulatory reduction on depressor responses after ergotamine. Pressor responses to adrenalin (0.05 or 0.1 cc. of 1:100,000) were completely reversed by intravenous injections of ergotamine, 1 mgm. per 100 grams body weight. The well marked depressor responses thus revealed were not significantly altered by reduction either of the splanchnic or of the peripheral circulation (12 expts.).

It has been shown that in the cat, after a small dose of ergotoxine which reduces but does not reverse the pressor response to adrenalin, clamping the aorta at the diaphragm to reduce the splanchnic circulation changes the pressor response to adrenalin into a pure depressor response (Rosenblueth and B. Cannon, 1933). A similar procedure in the rat did not significantly alter the pressor response to adrenalin previously reduced by the injection of ergotamine, 0.1 mgm. per 100 grams (4 expts.).

Discussion. Decrease of the pressor action of adrenalin in normal rats after splanchnic reduction, and increase after peripheral reduction, may be interpreted in two ways: first, that the rat, like the cat, has a preponderance of adrenergic vasodilators in the peripheral circulation; or second, that the rat, again like the cat, has a preponderance of vasoconstrictors in the splanchnic circulation as compared with the periphery. The fact that the depressor responses to small amounts of adrenalin in rats with the suprarenal medulla eliminated are more reduced by peripheral than by splanchnic reduction may be similarly interpreted. The results obtained after ergotamine treatment indicate, however, that only the latter explanation is applicable. The failure of either type of circulatory reduction to alter the depressor responses revealed by ergotamine, and the failure of splanchnic reduction to reveal a depressor response after a small dose of ergotamine, indicate that the rat has a significant distribution of adrenergic dilators to both splanchnic and peripheral regions, perhaps even an almost equal distribution.

Greater reduction of the depressor responses in suprarenalectomized rats after peripheral ligation may therefore be explained on the ground that the adrenergic vasodilators, released by the elimination of a depressant adrenin secretion (see Wyman and tum Suden, 1932), are working against a predominance of vasoconstrictors in the splanchnic area. After sufficient ergotamine treatment all vasoconstrictors are paralyzed, and the equal vasodepression in both areas in response to adrenalin shows significant distribution of dilators in both areas.

When injecting adrenalin into an animal with reduced circulation it must be borne in mind that the doses are relatively increased. This would help to account for the decrease of the depressor responses to small doses in suprarenalectomized animals by circulatory reduction where increase of adrenalin dosage brings more of the unaffected vasoconstrictors into action. It would also help to explain why the depressor responses,

after the vasoconstrictors are paralyzed by ergotamine, are unchanged by either type of circulatory reduction rather than equally reduced. In this case an increase of adrenalin dosage would produce increase of depression up to a certain point when acting on a given amount of the circulation served by adrenergic vasodilators.

The generally accepted distribution of sympathetic vasoconstrictors and vasodilators in the cat is ideally suited for a redistribution of blood to the skeletal muscles during conditions calling forth sympathetic activity and necessitating vigorous muscular activity. This is well known. The apparent difference of distribution in the rat may also have a biological significance. As compared with the cat, the rat might be called a "splanchnic animal"; that is, its bulk of visceral organs is large compared to its bulk of skeletal muscle. It may be that the need for a certain quota of sympathetic vasodilators for vasomotor readjustment under subemergency conditions necessitates their presence in the splanchnic circulation in the rat in larger amounts than in the cat, which has a relatively larger bulk of skeletal muscle to contain them. In order to offset their action during actual emergencies and to produce an effective redistribution of blood to skeletal muscles, the rat may in its evolution have developed an overemphasis of the differential depression of vasomotor mechanisms by adrenin—that is, greater depression of vasodepressor than of vasoconstrictor mechanisms by secreted adrenin. In a previous paper it was shown that such differential depression is not peculiar to the rat, being demonstrable under certain conditions in the cat; but in the rat it is easily demonstrated. Its possible significance in homeostasis was also discussed (Wyman and tum Suden, 1935). It would be of interest to investigate the situation in other animals of body build similar to that of the rat.

The demonstration of this apparent interspecific difference in the distribution of adrenergic vasodilators is of significance when one considers that general conclusions concerning the vascular physiology of mammals are prone to be drawn from the results of work on one or two species. Interspecific differences in physiological processes attending differences in body structure and in habits are known in connection with other organ systems. They may be expected also in the case of vascular physiology.

SUMMARY

1. Pressor responses to small amounts of adrenalin in normal rats are reduced about one half after reduction of the splanchnic circulation, and increased after reduction of the peripheral circulation. Pressor responses to larger doses of adrenalin are unchanged or slightly reduced by splanchnic reduction, and unchanged or somewhat increased by peripheral reduction.

2. Depressor responses to small doses of adrenalin in rats with the

suprarenal medulla eliminated are somewhat decreased after splanchnic reduction, and much decreased after peripheral reduction.

3. Depressor responses to adrenalin revealed in normal rats after treatment with ergotamine are unaltered by either type of circulatory reduction.

4. It is concluded that in the rat, as in the cat, adrenergic vasoconstrictors are more abundantly distributed to the splanchnic area as compared with the peripheral area; but that the rat, unlike the cat, has a significant distribution of adrenergic vasodilators to both regions. The biological significance of this apparent interspecific difference is discussed.

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VITAMIN C AND THE ADRENAL CORTICAL HORMONE[1]

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It has been accepted for a number of years that vitamin C was stored in the body only to a limited extent (1). Biologic tests have indicated that the rat, in contrast to other species such as the guinea pig, monkey, or man, is able to synthesize its own vitamin C (2). Evidence presented that "hexuronic acid" (later named "ascorbic acid"), isolated from the adrenal glands of the ox, was protective against scurvy when given to guinea pigs (3) opened up many new fields of investigation. Varying concentrations of ascorbic acid (vitamin C) have been proved to be present in the tissues of various species of animals by direct titration with the indophenol indicator (4). The results of this chemical mode of assay agree well with the biologic method.

Because of the constant presence of ascorbic acid in the adrenal glands of normal animals, many problems concerned with the relation between the adrenal cortical hormone (cortin) and vitamin C become apparent. Hypertrophy of the adrenal gland (5) has been found almost invariably in scorbutic guinea pigs. The relatively high concentration of ascorbic acid in the adrenal gland has been ascribed to the normal functioning of that organ (6). Evidence (6) from a brief experimental period of three to four weeks that ascorbic acid was not elaborated in the adrenal glands of those animals which are insusceptible to scurvy was not conclusive, for adrenalectomized rats are prone to have accessory adrenal glands (7). Adrenalectomized dogs, which can be kept alive with cortin, and in which, in the literature up to the present, no accessory cortical tissue has been reported to have been found, offered a better approach to the problem.

A study of the influence of the cortical hormone on nitrogen metabolism when adrenalectomized dogs were maintained on a diet free of vitamin C was undertaken. That the adrenal gland is concerned with nitrogen metabolism had already been demonstrated (8), and also that, following adrenalectomy, there is a marked increase of urea in the blood and a decreased excretion of nitrogen until the point of death is reached. Stud-

[1] The experiments presented in this paper were performed in the year 1933 to 1934. A preliminary report has been presented in Proc. Staff Meet. Mayo Clinic **9**: 312, 1934.

ies in this laboratory (9) on adrenalectomized dogs maintained on a diet with an adequate amount of vitamin C indicated that cortin had an influence on the nitrogen metabolism after administration of thyroxin. The balance of nitrogen metabolism tended to become negative in the absence of sufficient cortin, and the results indicated that nitrogen metabolism could be used as a criterion in the study of adrenal deficiency. Continuing these metabolic studies, if ascorbic acid furnished an essential link in the physiologic reactions concerned with cortin, then the absence of ascorbic acid in the diet might be reflected in the amount of cortin required to maintain the animal in normal condition. A failure in the physiologic processes involved in the utilization of cortin would be demonstrated by a change in the nitrogen metabolism if the animal were kept on a constant amount of cortin.

In addition to the studies on metabolism, the relationship between cortin and vitamin C was investigated. It had been reported (10) that the cortical hormone had a marked influence in delaying the onset of scurvy in guinea pigs. A critical survey of the experimental details indicated, however, that further study was necessary to clarify the connection between cortin and vitamin C.

Studies on the nitrogen metabolism of adrenalectomized dogs receiving a constant amount of cortin and maintained on a diet free of vitamin C. Two dogs, one male and one female, were used in this experiment. After bilateral adrenalectomy, which was done with the animals under ether anesthesia and employing sterile technic, the amount of cortical hormone necessary to keep the animal in normal condition was determined. The cortin had been prepared in this laboratory, and a sufficient supply, adequate for several months' use in order to have a uniform preparation, was stored in the icebox. Experiments with guinea pigs (the details of which will be discussed later), had conclusively shown that no ascorbic acid was present in these cortical extracts.

The basal diet used during the experimental period consisted of crackermeal (34 per cent), rolled oats (10 per cent) casein (14 per cent), bone ash (6 per cent), lard (8 per cent), cod liver oil (2 per cent), dried yeast (2 per cent), and distilled water (24 per cent). Guinea pigs fed on this basal diet died with symptoms of severe scurvy (four animals, scurvy score 16, survival period twenty-seven to thirty-two days, loss in weight 181 gm.). During the period of standardization, when the amount of cortin necessary to keep the animal in normal condition was being determined, an adequate amount of tomato juice in place of water was added to this basal diet as a source of vitamin C. The diet was analyzed for its nitrogen content, and the amount of food given to the animals was so weighed that the intake of nitrogen was constant during the duration of the experiment. A study of the nitrogen balance was carried out daily. All precautions

were taken, such as feeding and catheterizing the dogs at a definite time daily; urinary nitrogen was determined every day and the fecal nitrogen every ten days. Duplicate Macro-Kjeldahl determinations were made. Blood was withdrawn thrice weekly for the determination of urea nitrogen (van Slyke and Cullen modification of the Marshall method, as further modified at The Mayo Clinic). After twenty-eight days on this basal diet plus tomato juice, it was found possible to reduce the amount of cortin necessary to keep the level of blood urea between 30 and 40 mgm. per 100 cc. if 3 grams of sodium chloride were added to the diet. The dogs were maintained twenty more days on this diet and the amount of cortin necessary to maintain a normal condition was found to be 2 cc. daily (by subcutaneous injection).

During the experimental period tomato juice was omitted from the basal diet; everything else was unchanged. The dogs were kept in slightly positive nitrogen balance during the greater part of the period although, on four occasions, twice the daily dose of hormone had to be injected into the female dog for one day, the regular dose being resumed on the days following. On these occasions there were mild symptoms of adrenal deficiency, slight vomiting, elevation of blood urea, and the total nitrogen excreted exceeded the intake of nitrogen. One hundred and six days after the beginning of the experimental period the male dog was found dead. Necropsy revealed no signs of scurvy. The dog had gained 3 kgm., the amount of hormone injected daily had been sufficient, but a rectal temperature of 104°F. had developed the day before death which was ascribed to acute infection. After 161 days on the basal diet the other dog had gained 3.3 kgm. and, as it did not show any apparent signs of scurvy, the experiment was terminated. During the experiment the original preparation of the cortical hormone had been used up after 100 days, so that other preparations (one for forty-four and the other for seventeen days) had to be injected. The female dog was kept on the basal diet and used for other work on standardization, so that, after 230 days, it had gained 4.4 kgm. (fig. 1).

It is apparent from figure 1 that adrenalectomized dogs maintained on a diet free of vitamin C and receiving a constant amount of cortin show no marked deviations in the daily amount of nitrogen excreted. Any negative nitrogen balance that resulted at various intervals can be directly traced to a deficiency of cortin, as evidenced by refusal of the animal to eat food and by vomiting and so forth. These symptoms of deficiency were relieved by the addition of a larger amount of cortin for a day or two. The gain in weight and the absence of symptoms of scurvy after the long interval of time indicate that adrenalectomized dogs are undoubtedly able to synthesize their own vitamin C.

While this work was in progress, it was reported from another labora-

tory that adrenalectomized dogs used for standardization of cortin were kept free from scurvy on a vitamin C free diet (11). The present study confirms these results and was conducted under well defined conditions as regards the amount of cortin injected, sodium chloride in the diet and the intake of food. The recent results of other investigators (12), (13) who have been able to keep adrenalectomized dogs in normal condition for long intervals of time without the use of cortin but with adequate

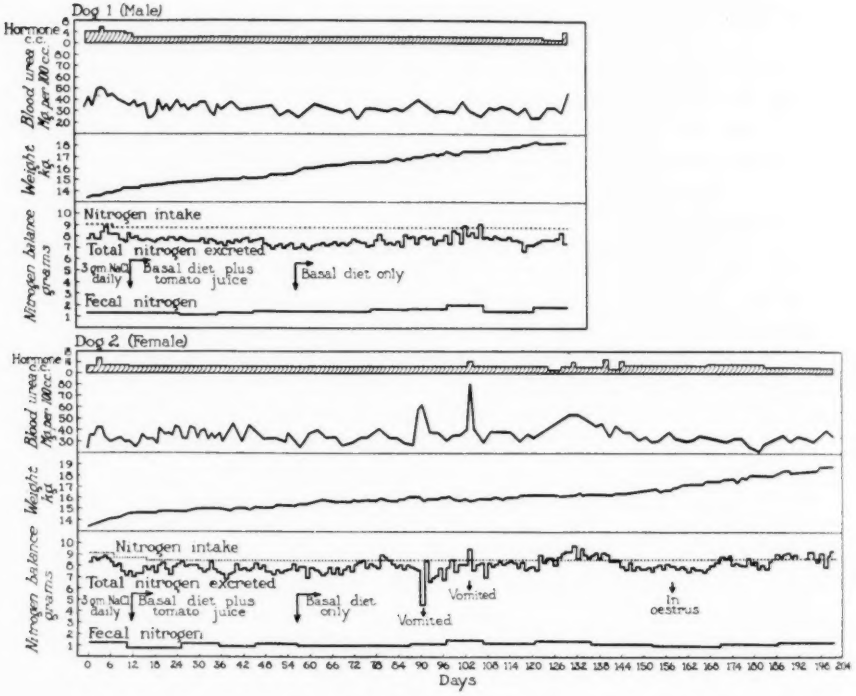

Fig. 1. Results of a study of the nitrogen balance of two adrenalectomized dogs on a vitamin C free diet receiving a constant amount of cortin.

amounts of various sodium salts in the diets, in no way alters the metabolism studies discussed. Under the conditions of the experiment a certain amount of cortin was necessary. All requirements for vitamin C must have been furnished by synthesis of the compound by the dogs.

Cortin and vitamin C. Young guinea pigs weighing 200 to 300 grams were used in the tests. The technic as to the preliminary care of the animals and as to diet, weighing, scoring and so forth described in the Sherman-Smith monograph (1), was followed. One cubic centimeter of

cod liver oil was given to each animal every week. Both the curative and preventive methods commonly employed in assaying vitamin C were used. In the curative method of assay (14) guinea pigs are placed on the basal diet and depleted of the vitamin C stored in their tissues. This period of depletion takes about two to three weeks and the substance to be tested is given to the animals when the weight for several successive days has been decreasing. Obviously, in this case, a somewhat larger dose of the substance is required, as compared to the preventive method wherein the animals receive smaller doses daily. The results of the curative method are, however, very striking and convincing, for if the substance is physiologically inactive, the downward trend in weight is not prevented and death follows, with the usual findings at necropsy.

TABLE 1

Response of guinea pigs to administration of cortin and vitamin C

NUMBER OF ANIMALS	DAILY DOSE	AVERAGE SCURVY SCORES*	AVERAGE GAIN OR LOSS IN WEIGHT	AVERAGE DAYS SURVIVAL
			gm.	
3	(Basal diet only)	13	−180	22 (18 to 33)
3	1 cc. cortin subcutaneously on twenty-second day	22	−119	33 (32 to 34)
2	0.25 mgm. ascorbic acid by mouth	8	+12	58†
3	0.25 mgm. ascorbic acid by mouth plus 0.2 cc. cortin subcutaneously	6	−44	58†
3	Mixture of 0.25 mgm. ascorbic acid and 0.2 cc. cortin injected subcutaneously	11	−79	33
2	0.5 cc. cortin subcutaneously from start		−145‡	

* Highest possible score 24.

† Etherized.

‡ Animals decidedly scorbutic on thirtieth day. Ascorbic acid (2 mgm.) given by mouth, following which the decline in weight was arrested and recovery resulted.

The cortin was taken from the preparation used in keeping the adrenalectomized dogs in normal condition, and it was therefore a potent preparation. The amount injected subcutaneously (0.2 cc. for an animal weighing 300 gm.) was adequate, based on the weight relationship (2 cc. of cortin for a dog weighing 15 kgm.) to prevent adrenal deficiency if the cortin was the limiting factor in delaying the onset of scurvy.

The ascorbic acid had been prepared from Hungarian red pepper (15) and had been kept sealed in vacuo in a glass vial. Solutions of ascorbic acid in ordinary distilled water were made up about twice a week, placed in vials, saturated with carbon dioxide gas, and kept in the icebox until

ready for administration. The solution was given by mouth by means of a graduated dropper and, in some instances, by subcutaneous injection (table 1, fig. 2).

It is evident from table 1 and figure 2 that cortin per se has no influence in delaying the onset of scurvy in guinea pigs. In the curative method of assay 1 cc. of cortin had no appreciable effect and the animals died with symptoms of scurvy and great loss of weight. The survival period was not prolonged and was within the usual time of eighteen to thirty-nine

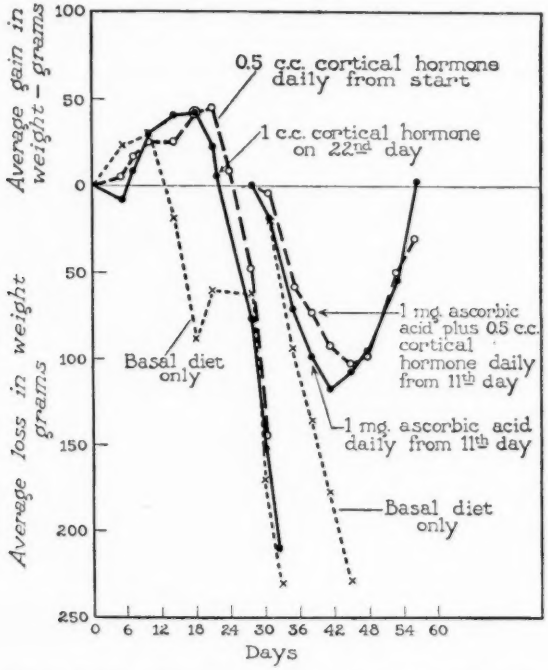
Average gain in weight - grams
Average loss in weight grams
100
50
0
50
100
150
200
250
0 6 12 18 24 30 36 42 48 54 60
Days
0.5 c.c. cortical hormone daily from start
1 c.c. cortical hormone on 22nd day
1 mg. ascorbic acid plus 0.5 c.c. cortical hormone daily from 11th day
1 mg. ascorbic acid daily from 11th day
Basal diet only
Basal diet only

Fig. 2. Effect of cortin and ascorbic acid on the decline in weight of scorbutic guinea pigs.

days. Similar results were obtained in another laboratory (11). Scorbutic animals recovered their vitality and recovered from loss in appetite and weight as readily when 1 mgm. of ascorbic acid was given by mouth as when the same amount of vitamin C was given with 1 cc. of cortin. Animals receiving 0.5 cc. of cortin daily began to lose weight on the eighteenth day, and on the thirtieth day were markedly scorbutic and lost on the average 145 grams. As in the previous case, these scorbutic animals recovered their vitality as readily with 2 mgm. of ascorbic acid as when the same amount of vitamin C was given in conjunction with cortin.

These tests indicate that vitamin C is the deciding factor in preventing scurvy.

In the preventive method of assay, with 0.25 mgm. of ascorbic acid by mouth (approximately equivalent to 0.7 cc. of orange juice) (10), there was no essential difference in the scurvy score, loss in weight, and so forth, as compared to those for animals receiving the cortin in addition to vitamin C. A mixture of cortin and ascorbic acid injected subcutaneously did not seem to give as favorable a response as when the same amount of vitamin was fed by mouth. With the preventive method of assay it has likewise been reported that cortin has no influence in retarding the onset of scurvy (16). Additional evidence (17) based on titration of the organs of guinea pigs given ascorbic acid alone and in conjunction with cortin has also been presented on this problem, so there can be scarcely any doubt that ascorbic acid alone is the limiting factor in preventing scurvy.

SUMMARY AND CONCLUSIONS

Adrenalectomized dogs maintained on a diet free of vitamin C did not develop scurvy. No changes in nitrogen metabolism ordinarily resulted when adrenalectomized dogs, receiving a constant amount of cortin, were maintained on a diet free of vitamin C. Marked fluctuations in the nitrogen metabolism depended on the amount of cortin injected.

Cortin has no appreciable effect in delaying the onset of scurvy, and ascorbic acid is the main factor in preventing scurvy in the gunea pig.

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LESIONS IN THE PANCREAS AND IN THE ANTERIOR HYPOPHYSIS WITH FATAL ACIDOSIS FOLLOWING PROLONGED INTRAVENOUS ADMINISTRATION OF GLUCOSE (IN DOGS)

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The physiologic processes by which normal animals utilize ordinary amounts of glucose have been the subject of many careful studies. However, the experiments designed to test these mechanisms have not ordinarily brought about harmful effects, hence the following report.

By flooding the normal animal with glucose a characteristic train of phenomena can be made to appear. These phenomena are caused, either directly or indirectly, by the presence of unusually large amounts of glucose in the body fluids, and may be interpreted as the consequence of overwhelming a normal mechanism.

It is obviously impossible to supply glucose to the tissues continuously for long periods at excessive rates, that is, at rates which exceed the utilization capacity of the normal animal, by any route other than the intravenous one. With the perfection of a method (1) for injecting unrestrained and unanesthetized dogs at constant rates for long periods of time, it has been possible to observe hitherto unrecognized metabolic and toxic effects of glucose injected by vein in dosages and over periods of time which overtax the capacity of the organism.

METHODS. By means of the method mentioned above, glucose was injected continuously into the jugular veins of normal female dogs. The glucose was given in water solution in concentrations of approximately 50 per cent. The rates of injection varied between 0.7 and 4.5 grams of glucose per kilogram of body weight per hour. No food was given, but water was allowed as desired. In all, twenty-two experiments were finished.

Blood samples were drawn from the saphenous vein. The blood was analyzed for carbon dioxide combining capacity by the method of Van Slyke and Cullen and for sugar according to Folin and Wu. The urine was collected by catheterization at definite intervals. It was analyzed for sugar according to Folin and Berglund, and for total nitrogen by the method of Kjeldahl.

The post-mortem and histologic examinations were made by accepted methods.

GENERAL OBSERVATIONS. The general behavior of the dogs varied considerably with the individual experiment and rate of glucose administration. During the first and longest period of the injection they usually appeared normal except for variable degrees of thirst and polyuria. Later they frequently appeared weak. During the final (premortal) hours they became listless and stuporous, usually breathing heavily and rapidly, finally passing into unconsciousness which was sometimes punctuated by tonic and clonic movements in the last phase. All our experiments ended between the first and seventh days, the earlier with the higher administration rates, as a rule. There were no significant changes in the body weight.

THE ANATOMIC CHANGES. The anatomic changes, although fairly constant, varied with the individual dog as well as with the rate of glucose injection and the length of the experiment. In general, however, the greatest changes followed the largest dosages.

As a rule, all the tissues were moderately hyperemic. The serous membranes and the meninges usually were obviously congested with blood. But neither dehydration nor edema of the body tissues in general was noted. Except for the changes described here, the tissues seemed quite normal.

The pituitary body. The anterior lobe of the pituitary body was usually very markedly hyperemic (fig. 3), and apparently swollen and softened. Microscopically, hyperemia was manifest in the anterior lobe, and the number of eosinophilic cells appeared to be increased. The posterior lobe was only slightly congested (fig. 4).

The heart and lungs. The heart was often found in systole. Grossly and microscopically, the myocardium was moderately hyperemic, and stains for fat and glycogen revealed nothing remarkable. The lungs were usually pink and moderately congested, but not otherwise abnormal.

The liver. The liver was often massively enlarged, sometimes reaching the pelvic brim. It was pale and very friable. In one animal its surface was covered with bloody blebs and its parenchyma spotted with hemorrhage. Microscopically, the hepatic cells were swollen with pale cytoplasm. Their nuclei were dense. The fat stain (Sudan III) disclosed no abnormal amount of fat. The glycogen stain (Best's) was strongly positive.

The pancreas. The pancreas was often found in a remarkable state. It was moderately congested at least, but usually contained gross hemorrhages which varied in size and number from discrete areas (fig. 2) to large suggillations which obliterated the organ (fig. 1). In two instances the pancreas was swollen and glairy from edema (which also spread into

the adjoining mesentery). Microscopically, there were marked inter- and intralobular congestion and frank hemorrhage into the parenchyma. The acinar tissue often showed retrogressive changes, and in many sections islet tissue could not be found (hidden by hemorrhage?) (fig. 5). In the

Fig. 1

Fig. 2

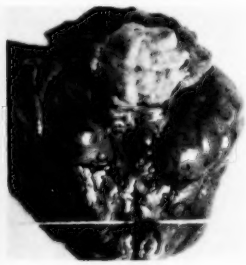

Fig. 3

Fig. 1. Photograph of pancreas. The organ is entirely filled with extravasated blood.

Fig. 2. Photograph of pancreas. There are numerous hemorrhages in the organ.

Fig. 3. Photograph of the base of the brain showing marked congestion of the hypophysis. Note also the meningeal hyperemia.

Fig. 4

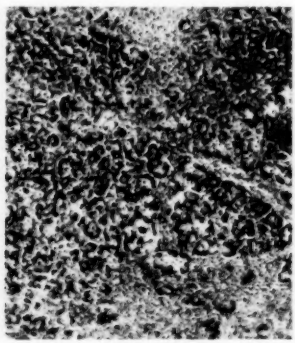

Fig. 5

Fig. 6

Fig. 4. Sagittal section of the hypophysis, showing hyperemia and hemorrhage in anterior lobe.

Fig. 5. Microphotograph of pancreas. There is much hemorrhage into the parenchyma. Acinar tissue undergoing retrogressive changes. Islet tissue scarce.

Fig. 6. Microphotograph of cross-section of suprarenal gland. Moderate congestion in middle zone of the cortex.

cases showing the least change, the islet cells appeared to have taken the eosin stain more deeply than in the normal pancreas. In those most profoundly changed, no pancreatic tissue of either form could be found; the pancreas was apparently gangrenous at the time of death of the animal.

The suprarenal glands. The suprarenal glands showed no marked or constant changes. In some there was hyperemia both grossly and microscopically in the middle zone of the cortex (fig. 6). In many the medulla, which is normally lightly stained (by hematoxylin and eosin), was stained a brownish-green color.

The kidneys. The kidneys were usually dark and congested. Microscopically, the convoluted tubules showed retrogressive changes and increased fat content. The glomeruli were not changed.

The stomach and intestines. Except for moderate congestion of its serosa and mucosa, the gut was not abnormal.

The skeletal muscles. The musculature presented no gross changes. Glycogen was present in normal amounts.

The thyroid and parathyroid glands, the ovaries and Fallopian tubes were normal in all the animals.

THE CHEMICAL CHANGES. The chemical changes also varied somewhat with the individual animal as well as with the rate of glucose "utilization." The term "utilization" is here used as Woodyatt has consistently used it to indicate the capacity of the animal to make use of glucose constantly supplied. It is the difference between the amounts supplied and excreted when the supply is known to be constant, as in the present experiments.

The utilization of glucose by our animals is illustrated by the graph in figure 7. These instances were chosen to show the early rise in utilization and the duration of life with several dosages of glucose. It is apparent from the graph that animals receiving glucose at rates within their capacity to metabolize and store (I, II and III) lived much longer than those which received and hence excreted larger amounts of the sugar (IV–VII).

The changes appearing in the blood and urine during typical experiments are shown in tables 1 and 2. Table 1 shows the findings with a relatively low rate of glucose administration; table 2 shows the effects of an administration rate approximately three times as great. Both experiments demonstrate three distinct phases which were quite typical of all experiments. During the first phase there is a rise in utilization, an increase in the excretion of water and nitrogen, usually glycosuria, a rise in the blood sugar level and an irregular fall in the carbon dioxide combining power of the blood. During the middle phase, the length of which depends largely upon the dosage of glucose, the utilization remains high, but the diuresis, nitrogen excretion, glycosuria and blood sugar level tend to fall to a constant level. The carbon dioxide combining power of the blood tends to become gradually lower. The last phase is characterized by a rapid fall in the carbon dioxide combining capacity and a rise in the sugar level of the blood, and a rapid fall in water, nitrogen and sugar excretion. In the last few pre-mortal hours the "utilization" is obviously fictitious. The last period usually begins suddenly and ends shortly in the death of the animal.

The urine excreted during the experiments was neutral or faintly acid to litmus. The ammonia content was normal. Qualitative tests for ketone bodies were uniformly negative.

COMMENT. These experiments, although perhaps not within physiological limits, yield some interesting facts and permit some degree of interpretation. They show that the continuous administration of glucose even at rates just within the limits of tolerance, that is, not causing glycosuria, is always fatal. Further, they show that higher injection rates, causing glycosuria, are more rapidly fatal. The mechanism leading to death would appear to be a non-ketogenic acidosis associated with profound congestive and hemorrhagic changes particularly involving the pancreas and anterior hypophysis.

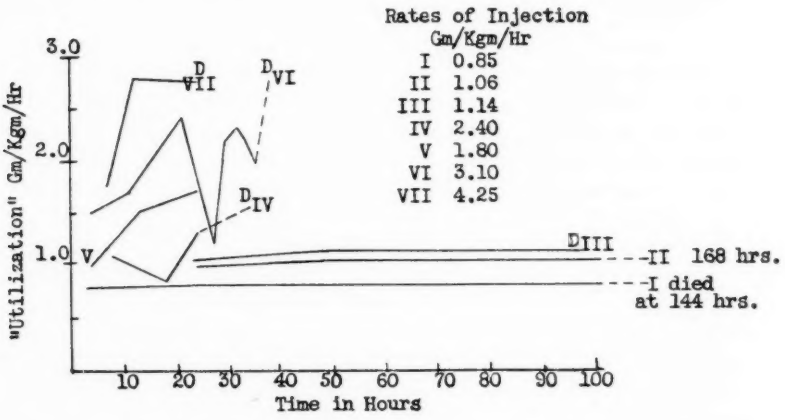

Fig. 7. Showing the duration of life and the utilization with several rates of injection. Dogs I and II continued to utilize at the rates shown until they died at 144 and 168 hours respectively. (*D*) means the death of the animal.

The observed anatomic changes, even more than the chemical data, indicate that the injection of glucose imposes a great strain upon the metabolism of the animal and may cause irreparable injury to the pancreas and perhaps to the hypophysis. The selective changes involving the pancreas and hypophysis seem remarkable and are difficult to interpret. The experiments of Houssay and others showing a functional relationship between the pancreas and the hypophysis in sugar metabolism suggest a physiological significance of this phenomenon, but the nature of it is still obscure. The gravity of the anatomic changes explains the failure of our attempts to revive the animals in the last period of the experiments through intravenous injections of physiological saline, sodium bicarbonate and adrenalin solutions. An evaluation of the anatomic

changes must consider the congested state of all the tissues as well as the more marked gross changes in the pancreas and hypophysis.

An interpretation of the chemical data here presented is also difficult, particularly in the absence of determinations of blood, tissue and urine anions and cations. Although it has been shown that diuresis will lower

TABLE 1

Constant intravenous glucose injection

Dog 11, non-gravid female, weight 11.5 kgm. Rate of injection: 13.1 gm./hr. or 1.14 gm./kgm./hr. or 25.7 cc. of 50.3 per cent glucose per hour.

HOUR OF EXPT.	HOURS IN PERIOD	URINE	NITROGEN EXCRETED	GLUCOSE EXCRETED	GLUCOSE "UTILIZATION" (CALCULATED)	BLOOD SUGAR	BLOOD CO_2 CAPACITY
		cc./hr.	*gm./hr.*	*gm./hr.*	*gm./kgm./hr.*	*per cent*	*vol. per cent*
0						0.12	41.9
25	25	35.4	0.188	0.94	1.06	0.13	37.9
48	23	24.8	0.104	0.022	1.14	0.13	48.7
$72\frac{3}{4}$	$24\frac{3}{4}$	54.1	0.136	0.027	1.14	0.16	33.5
$93\frac{3}{4}$	21	16.4	0.053	0.026	(1.14)	0.62	9.0

TABLE 2

Constant intravenous glucose injection

Dog 26, non-gravid female, weight 11.7 kgm. Rate of injection: 37.0 gm./hr. or 3.1 gm./kgm./hr. or 58.3 cc. of 62.8 per cent glucose per hour.

HOUR OF EXPT.	HOURS IN PERIOD	URINE	NITROGEN EXCRETED	GLUCOSE EXCRETED	GLUCOSE "UTILIZATION" (CALCULATED)	BLOOD SUGAR	BLOOD CO_2 CAPACITY
		cc./hr	*gm./hr.*	*gm./hr.*	*gm./kgm./hr.*	*per cent*	*vol. per cent*
0						0.09	52.2
4	4	292.5	0.210	22.27	1.26	0.85	39.3
11	7	197.0	0.224	17.06	1.71	0.54	38.4
21	10	104.0	0.105	8.42	2.44	0.35	29.8
24	3	123.3	0.096	15.80	1.81	0.45	33.0
27	3	126.6	0.053	22.37	1.25	0.49	33.8
29	2	130.0	0.040	10.25	2.29	0.46	28.4
31	2	145.0	0.040	10.36	2.27	0.48	28.2
33	2	155.0	0.040	10.54	2.27	0.52	25.7
35	2	155.0	0.030	12.08	2.13	0.97	23.8
$38\frac{1}{6}$	$3\frac{1}{6}$	69.9	0.00	3.19	(3.03)	1.30	17.8

the alkali reserve of blood (2), (3), diuresis alone cannot account for the rapid fall in alkali reserve noted in the acute experiments of Boyd, Hines and Leese (4), and of Foshay (5). Furthermore, the most rapid decrease in the alkali reserve was accompanied by a decrease in the output of urine in our own experiments. The numerous and heterogeneous reports in

the literature on the behavior of blood, tissue and urine cations and anions in normal animals treated with glucose, insulin and adrenalin suggest that an acidic substance appears when glucose is being metabolized. From this point of view the changes observed may be considered the result of an acid intoxication, mild during the early parts of the experiments when the hypothetical acidic substances can be disposed of by metabolism or neutralization, grave when these mechanisms fail. The whole matter is complicated by the large amounts of glucose constantly present, which, by overloading tissue cells through storage (liver) and by changing the osmotic relationships in body fluids, exert rather non-specific effects not calculable in terms of normal processes. The early increased nitrogen excretion can be attributed to "washing out," because the later, lower nitrogen excretion excludes tissue breakdown as an important factor.

Our data corroborate the conclusions of Felsher and Woodyatt (6) who found a fairly sharp limit in the tolerance of normal dogs to glucose. These authors also noted that the tolerance varies somewhat with the individual animal, in their experiments ranging between 0.8 and 1.4 gram per kilogram of body weight per hour.

SUMMARY

1. Prolonged and continuous administration of large amounts of glucose by vein to normal dogs results in:

a. Early and sustained improvement in the capacity to make use of the administered sugar, failing terminally.

b. Remarkable hepatic storage of glycogen.

c. Progressive depletion of the alkali reserve, resulting ultimately in fatal acidosis.

d. Generalized mild congestive changes in all viscera.

e. Specific intense hemorrhage into and destruction of the pancreas and the anterior lobe of the hypophysis.

2. Convincing interpretation of these phenomena is not possible from the presented data. The formation of excessive amounts of an unidentified acidic intermediate product of glucose metabolism is surmised.

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THE EXPERIMENTAL INDUCTION OF OESTRUS (SEXUAL RECEPTIVITY) IN THE NORMAL AND OVARIECTOMIZED GUINEA PIG[1]

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Notwithstanding the reports that spayed mice (Allen et al., 1924; Marrian and Parkes, 1930; and Wiesner and Mirskaia, 1930), rats (Allen et al., 1924; Hemmingsen, 1933), rabbits (Lacassagne and Gricouroff, 1925), and dogs (Kunde, D'Amour, Carlson and Gustavson, 1930) copulate following the injection of either follicular fluid or oestrin, an analysis of the mating behavior described for the guinea pig (Young, Dempsey and Myers, 1935a, b) has yielded several lines of evidence indicating that oestrin alone is not sufficient to produce mating behavior.

When it was learned that the one ovarian change which is associated with the beginning of heat is the beginning of the preovulatory swelling (Myers, Young and Dempsey, 1935), and that injection of the luteinizing hormone causes the preovulatory swelling (Foster and Hisaw, 1935), it seemed the luteinizing hormone might be the extra-ovarian factor we had postulated to be associated with oestrin in causing heat. Evidence will be presented below which partially supports this hypothesis. It now appears, though, that the luteinizing hormone does not directly produce heat, but stimulates the preovulatory swelling, ovulation and the production of progesterone, and it is this latter substance acting synergistically with oestrin that causes heat. The experiments upon which this conclusion is based are described in the following sections.

The injection of oestrin preparations into spayed and normal females. When water soluble theelin was injected subcutaneously into spayed females, heat followed only three of fifty-seven injections after latent periods of 33 to 42 hours (table 1). This low percentage of positive results is not attributable to the injection of insufficient quantities, since a four-fold increase in the minimal effective dose did not produce heat.

Theelin-in-oil was more efficacious in producing heat than water soluble theelin, seventeen of fifty-four injections being followed by oestrous periods of one to twenty-one hours. Still better results were obtained

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with dihydroxyoestrin benzoate (Progynon-B, Schering). Eleven of seventeen injections were followed by heat periods of six to seventy-seven hours. Two unusual features of the heat induced by this latter substance are its excessive length (sixty-six and seventy-seven hours) in two instances and the occurrence of a "split oestrus" (Young, Dempsey and Myers, 1935a, p. 323) in four instances.

It was thought the administration of several small injections followed by a large injection might more nearly simulate the normal changes in oestrin level in the animal. Accordingly, five animals were injected twice daily with 12.5 I.U. theelin-in-oil, followed by 1000 I.U. four days after the first injection, and eight animals were injected four times daily with 10 I.U. followed by 1000 I.U. five days after the first injection. The results obtained were not different. Altogether, five animals came into heat. Of these, two came into heat while the small injections were being made, but not later after the large quantity was injected; two came into heat once while the small quantities were being injected and again after the large injection, and one came into heat only after the large injection. Since only five of thirteen animals came into heat, the administration of several small injections followed by a large injection is no more effective than a single large injection.

The injection of theelin into normal animals was undertaken with the thought that the normal oestrus might be lengthened by supplying additional theelin. The unexpected result was obtained that animals with intact ovaries respond to such injections less frequently than spayed individuals. Only two of ten animals injected with theelin-in-oil at the beginning or the end of heat came into heat again, and in no case was the normal oestrus longer than the previous normal periods.

Since it was thought the absorption time of the oil soluble theelin might be so long that it would defeat our purpose of augmenting the animal's own supply of theelin, another series of injections was made with water soluble theelin (table 2). Nine animals were injected with 25 R.U. every hour for four hours, beginning at the onset of oestrus and continuing until at least two hours after the end of oestrus, and three animals were injected with 25 R.U. every hour for four hours, beginning with the end of oestrus. Again, the heat period was not lengthened, the average for the nine animals injected from the beginning of oestrus being 6.5 hours which is 1.5 hour shorter than the average for the colony.

A last group of normal animals was composed of six injected with 1000 I.U. theelin-in-oil on the eighth day of the dioestrum and seven injected on the twelfth, thirteenth or fourteenth day. Only one animal, injected on the eighth day, came into heat after a latent period of seventeen hours. This was the only time a latent period of less than twenty-eight hours followed the injection of oestrin. Examination of the ovaries of the twelve

day animals forty-eight hours after the injection revealed normal large follicles but no ovulation points. The next oestrus occurred either as though nothing had happened (2 cases), or following a delay of three (4 cases) or four (1 case) days. Apparently, the action of theelin late in the cycle slightly retards, rather than hastens, the appearance of the next oestrus.

When the animals injected with oestrin preparations are considered as

TABLE 1

Effect of oestrin preparations in causing heat in spayed animals

PREPARATION	AMOUNT	NUMBER OF ANIMALS	NUMBER OF INJECTIONS	INJECTIONS FOLLOWED BY HEAT		INJECTIONS FOLLOWED BY MOUNTING	LATENT PERIOD	LENGTH OF HEAT
				Number	Percent			
							hours	*hours*
Theelin (water sol)	100 R.U. or more	24	57	3	5.3	15	33–42	1–12
Theelin (oil sol)	1000 I.U.	30	54	17	31.0	12	35.3	1–21
Progynon-B	1000 I.U.	17	17	11	65.0	1	33.4	6–77

TABLE 2

Effect of oestrin preparations in causing heat in normal animals

PREPARATION	AMOUNT	STAGE IN CYCLE	NUMBER OF ANIMALS	INJECTIONS FOLLOWED BY HEAT	INJECTIONS FOLLOWED BY MOUNTING	LATENT PERIOD
						hours
Theelin (water sol)	100 R.U. or more	Onset of oestrus	9	0	1	
	100 R.U. or more	End of oestrus	3	0	1	
Theelin (oil sol)	1000 I.U.	Onset of oestrus	7	1	0	40.5
	1000 I.U.	End of oestrus	3	1	0	31.5
	1000 I.U.	8th day	6	1	0	17.0
	1000 I.U.	12th day	7	0	0	

a whole, the following features are noted. 1. An injection effective at one time may be ineffective at another time. 2. Divided injections of small quantities of oestrin frequently are quite as effective as large quantities. 3. Normal animals respond to oestrin less frequently than spayed animals. 4. When heat occurs following a single injection of water-soluble theelin, theelin-in-oil, or dihydroxyoestrin benzoate, the length of the latent period is the only reasonably constant feature. In no instance

following thirty-one effective injections into spayed animals was the latent period less than twenty-eight or more than forty-five hours.

Altogether, the data given above emphasize that the results following oestrin injections are characterized by irregularity and are difficult to reconcile with the view that oestrin alone is responsible for the production of heat.

The injection of luteinizing hormone into normal and spayed females. When 0.2 gram equivalent of sheep luteinizing hormone extracted according to the method of Fevold, Hisaw, Hellbaum and Hertz (1933) was injected intravenously into the marginal ear vein of twenty-four animals on the twelfth to fourteenth day of the cycle, nineteen came into heat after an average latent period of 5.8 hours (table 3), a result which shortened the dioestrum an average of 2.6 days. The heat period produced averaged only 5.6 hours in length, and is therefore shorter than the normal oestrus

TABLE 3

Effect of intravenous injections of luteinizing hormone preparations in causing heat in normal animals

PREPARATION	AMOUNT	STAGE IN CYCLE	NUMBER OF ANIMALS	INJECTIONS FOLLOWED BY HEAT	LATENT PERIOD
					hours
Sheep L.H.	0.2 g. eq.	12th–15th day	24	19	5.8
Mare serum L.H.	0.4 g. eq.	12th–15th day	6	4	6.1
Antophysin	25–100 R.U.	12th–15th day	7	5	6.4
Sheep L.H.	0.2 g. eq.	8th day	4	0	
Sheep L.H.	0.2 g. eq.	4th day	4	0	
Sheep L.H.	0.2 g. eq.	End of oestrus	3	0	

shown previously by these animals which averaged 7.9 hours. Laparotomy in fifteen animals and histological examination from seven cases has shown that ovulation and luteinization occur following this treatment.

Both heat and ovulation were also produced at this time in four of six cases by a luteinizing hormone fraction of pregnant mare serum extracted according to Fevold's method, and in five of seven cases by the pregnancy urine concentrate, Antophysin. Since these substances contain no lactogenic hormone (Riddle, Bates and Dykshorn, 1932), growth hormone, adrenotropic factor or thyreotropic factor (Evans, Meyer and Simpson, 1933), the above results probably can be attributed to the luteinizing hormone alone.

When the luteinizing hormone was injected into four animals on the fourth day and four animals on the eighth day of the dioestrum, and into three animals at the end of heat, oestrus did not follow. Sexual receptivity, then, can be produced by administration of the luteinizing hormone only when there are follicles in the ovary mature enough to be ovulated.

The next normal oestrus following the injection of the luteinizer occurred nine to fifteen days later, the average being 13.2 days. This suggests that the lutein tissue formed following the injections was hypofunctional, since animals from which the corpora lutea have been removed soon after the end of oestrus return to heat and ovulate in about this time (Loeb, 1911; and unpublished data from this laboratory).

The failure of luteinizing hormone to produce oestrus early in the cycle suggested that possibly sufficient oestrin to condition the animal had not been produced before the twelfth day. However, when two six-day and two eight-day animals were given 1000 I.U. theelin each and forty-eight hours later were injected with luteinizing hormone, heat did not occur. Since the presence of a functional corpus luteum at this time might counteract the effect of the theelin, spayed animals were injected with 1000 I.U. theelin, and forty-eight hours (5 animals) and twenty-four hours (5 animals) later were injected intravenously with luteinizing hormone. Again, heat

TABLE 4

Effect of 40 I.U. oestrin followed by 0.2 I.U. progesterone in causing heat in spayed animals

TIME BETWEEN INJECTIONS	NUMBER OF ANIMALS	INJECTIONS FOLLOWED BY HEAT	INJECTIONS FOLLOWED BY MOUNTING	LATENT PERIOD
hours				*hours*
0	4	0	0	
12	8	2	0	9.5
24	8	6	1	6.6
36	3	3	1	4.3
48	24	24	5	4.8

did not occur. Likewise, when four animals, all of which previously had responded to injections of oestrin alone were given 1000 I.U. dihydroxyoestrin benzoate and twelve hours later were injected with luteinizer, heat occurred in only two animals after latent periods of 30 and 45 hours, the usual latent period for oestrin. Apparently, therefore, the combination of oestrin and luteinizing hormone can neither produce oestrus regularly nor shorten the latent period of oestrin. This indicates that the action of the luteinizer in producing oestrus is mediated through the ovary, and since the luteinizing hormone causes the formation of the corpus luteum, the next attempts to produce oestrus involved injections of the corpus luteum hormone.

The injection of progesterone into spayed and normal females. Because all the known progesterone reactions occur only after an initial treatment with oestrin, a similar procedure was employed. In a first series twenty-eight spayed females were injected subcutaneously with 40 I.U. theelin

over a period of forty-eight hours, followed by 0.05 to 0.2 I.U. of progesterone (Proluton, Schering). The twenty-four animals which received the larger amount came into heat following latent periods of three to nine hours, the average being 4.8 hours. One of the animals receiving 0.1 I.U. came into heat and 0.05 I.U. was not effective in either of two cases.

To determine whether an initial treatment with oestrin is necessary, four spayed animals were injected with 0.2 I.U. progesterone alone. Not one of the four came into heat. Theelin injections were then followed 0, 12, 24 and 36 hours later by progesterone (table 4). No one of four animals injected with the two preparations simultaneously came into heat. Of the others, two of eight in which the injections were twelve hours apart, six out of eight in which the injections were twenty-four hours apart, and all in which the injections were thirty-six or more hours apart came into heat. Therefore, an initial treatment with oestrin at least twelve hours prior to the injection of progesterone is necessary.

A corollary to the above developed when progesterone was injected at the end of oestrus. Whether the oestrus was normal (3 cases), induced by oestrin alone in spayed animals (2 cases) or induced by oestrin and progesterone in spayed animals (2 cases), there was no return of heat. Apparently, once oestrus has been evoked, the animal must again be conditioned before heat can occur.

In the case of some of the oestrin-progesterone reactions, a rather definite quantitative relationship has been shown to be necessary (Hisaw and Leonard, 1930). However, when four spayed animals were injected with 1000 I.U. theelin and forty-eight hours later with 0.2 I.U. progesterone, all four came into heat. Likewise, when four injections of 250 I.U. each were made over the forty-eight hour period, followed by 0.2 I.U. progesterone, oestrus occurred. Thus, the result was no different from that obtained following the smaller amounts of oestrin administered in the earlier experiments, indicating that there is considerable latitude in the quantitative relationships of the reaction.

Fourteen normal animals were injected with progesterone alone on the twelfth to fifteenth days of the dioestrum. Of those injected on the fourteenth to fifteenth days, seven of ten came into heat. Of those injected on the twelfth and thirteenth day, unlike similar animals injected with luteinizing hormone, heat occurred in only one of four animals. When, however, such animals were injected with 40 I.U. theelin and thirty-six hours later with progesterone, oestrus followed in four of six animals. Laparotomies following these induced heat periods showed that ovulation had not occurred in any of the nine animals examined. Despite the operation, six of the nine animals came into heat again spontaneously four or five days after the experimental oestrus and second laparotomies in three animals showed that ovulation had occurred then. In these cases, then, it would seem that the substance which normally is produced at ovulation

and which causes oestrus was introduced into suitably conditioned animals and produced sexual receptivity without ovulation.

On the basis of the results obtained from these experiments, it seems likely that progesterone is involved very directly in the production of sexual receptivity in the guinea pig. The chain of events is thought to be approximately as follows: The rise in the oestrin level which occurs toward the end of the cycle conditions the animal for oestrus. The appearance of oestrus itself, however, depends upon the subsequent action of progesterone which is produced in the still unruptured follicle after it has been acted upon by the luteinizing hormone of the hypophysis.

Discussion. While this investigation was in progress, Witschi and Pfeiffer (1935) reported that mating reactions can be induced in rats by injections of luteinizing hormone, provided mature follicles are present. Since ovulation also occurred, it was not clear to them whether the mating instincts were aroused directly by the luteinizer or by concomitant changes occurring in the ovary. The production of sexual receptivity in spayed animals by injections of oestrin and progesterone and our failure to evoke mating reactions by injections of luteinizing hormone and oestrin would seem to answer this question as far as the guinea pig is concerned. The rôle of the luteinizer is not direct, but acting upon the ovary, it causes the production of progesterone which brings the animal into heat.

Our failure to hasten ovulation in normal animals injected with oestrin or progesterone, or both, on the twelfth to fifteenth days of the cycle has caused us to question the hypothesis (Lane, 1935) that the release of the luteinizer is effected when an increased level of oestrin acts upon the pituitary. This failure to hasten ovulation can not be attributed to the immaturity of the follicle, since ovulation can be produced at this time by injections of luteinizer. In addition, the approach of darkness, an environmental factor, influences the time of oestrus (Dempsey, Myers, Young and Jennison, 1934) and therefore, it would seem, the time of the release of the luteinizer.

The current theories regarding the relationships between the pituitary and the rest of the endocrine system render caution advisable in assigning the cause of oestrus to the hormonal mechanism we have described. For example, we do not know why oestrin alone brings some animals into heat. A possibility is that there is a conversion of oestrin to progesterone in the animal. Nevertheless, the data which indicate that oestrus is caused by the action of progesterone after the animal has been conditioned are felt to clarify the ultimate cause more than the hypothesis that oestrin alone is responsible. In addition, a procedure that can be depended upon to bring females of at least one species into heat should be useful as a point of departure from which studies of the complete regulatory mechanism can be continued.

Indispensable assistance in observing the animals has been given us

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SUMMARY

1. Only 14 per cent of injections of 1000 I.U. theelin in 21 normal females and 31 per cent of 54 injections in 30 spayed females were followed by sexual receptivity. The latent period averaged 35.3 hours.

2. Intravenous injections of 0.2 gram equivalent of sheep luteinizing hormone produced heat and ovulation in 19 of 24 cases when injected into normal animals on the 12th to 15th days of the dioestrum, but were ineffective earlier in the cycle in 15 cases. The latent period averaged 5.8 hours. Similar injections of luteinizing hormone into spayed females were ineffective and the combination of oestrin and luteinizing hormone did not enhance the efficiency of the oestrin alone in 15 cases.

3. Injections of 40 I.U. or more of oestrin, followed 36 to 48 hours later by 0.2 I.U. progesterone invariably produced heat in 24 spayed females. The latent period averaged 4.8 hours after the injection of the progesterone. Seven of nine normal animals injected with progesterone alone on the 14th or 15th day of the cycle came into heat 5 to 10 hours after the injection, but did not ovulate.

4. On the basis of these data, it is postulated for the guinea pig that after conditioning with oestrin, oestrus is caused by the action of progesterone, produced under the influence of the luteinizing hormone during the preovulatory enlargement of the follicle.

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IS A PORTION OF THE PANCREATIC SECRETORY RESPONSE TO A MEAL DUE TO THE ABSORPTION OF DIGESTED FOOD PRODUCTS?

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It is well established that the extrinsic nerves of the pancreas influence the secretion of pancreatic juice and that the hormone secretin is predominantly concerned in the genesis of the post-cibum secretion. Since Kim and Ivy (1) found recently that certain circulating split-protein products (liver extract, the 70 per cent alcohol soluble and the 95 per cent alcohol insoluble fraction) free of vasodepressins stimulate gastric secretion and *may* serve in part as the humoral agency concerned in the intestinal phase of gastric secretion, we decided to ascertain if certain products which enter the circulation after a meal may stimulate pancreatic secretion.

The only observations known to us which bear directly on this problem are those of Frouin (2) and Arai (3), in which certain amino acids were injected parenterally without stimulating the pancreas.

Split-protein products. We first ascertained if the intravenous injection of a solution of split-protein products at a slow rate would stimulate the pancreas. We chose liver extract which was used in the experiments on gastric secretion referred to above (1) and Witte's peptone. Liver extract was chosen because the mode of its action in pernicious anemia indicates that an amino acid complex present in it is absorbed from the intestine. Witte's peptone was chosen because it has been used rather extensively to stimulate pancreatic secretion (3, 4).

Liver extract. Under barbital anesthesia the pancreatic ducts of six dogs were cannulated. The continuous secretion for thirty minutes was measured. Then, 4.2 grams of liver extract (no. 343, Lilly & Co.) powder (that fraction soluble in 70 per cent alcohol and insoluble in 95 per cent alcohol) dissolved in 50 cc. of 0.9 per cent sodium chloride solution, were injected intravenously during a period of 20 minutes, and the secretion measured. After a control period of thirty minutes, the same quantity of liver extract from which the vaso-depressins had been previously removed (1), was likewise injected. After another 30 minute period had

elapsed, a threshold dose of secretin was given and the resulting quantity of pancreatic juice was measured. This was later followed by an injection of the same dose of secretin plus untreated liver extract. The pylorus was ligated to prevent a false response from occurring due to the passage of gastric juice into the intestine.

The results were so consistent as not to require tabulation and may be summarized as follows: The untreated liver extract containing some vaso-depressor substances caused a two-fold increase in the continuous secretion, but the liver extract freed of vaso-depressor activity was inert. The liver extract was added to secretin to ascertain if the two acted synergistically. It was found that they did not, the flow of juice being only approximately doubled. This finding is not physiologically significant, since it is well known that the presence of a little vasodilatin in a secretin preparation augments the flow of pancreatic juice resulting from secretin alone. The foregoing experiment was repeated on seven dogs having a "chronic" pancreatic fistula with the same results.

Witte's peptone. It is certain that the intravenous injection of peptone (20–30 mgm. per kilo), like histamine (0.5–1.0 mgm.), causes a slight augmentation of the flow of pancreatic juice (0–20 drops). Gley (6) thought the action of peptone was selective for the pancreas. Others (4, 5) interpreted the augmentation of flow as being due to the action of histamine or an histamine-like substance. Sodre and Stodel (7) extracted Witte's peptone with alcohol and found the residue as well as the extract showed some potency.

In our experiments (forty) performed on "acute" fistula dogs, four different peptones were used, namely, *a*, Witte's peptone (fibrin), which always lowered blood pressure in varying degrees and stimulated pancreatic secretion; *b*, Wilson's peptone made by the peptic digestion of meat, which generally caused a slight fall of blood pressure followed by a rise and stimulated pancreatic secretion in the unanesthetized but not in the anesthetized dog; *c*, Wilson's peptone made from gelatine by pancreatic digestion, which raised the blood pressure slightly and had no effect on pancreatic secretion; and *d*, Wilson's peptone made from casein by pancreatic digestion, which caused only a slightly perceptible fall in blood pressure and no stimulation of pancreatic secretion. The peptones were given in 30 mgm. per kilo doses in normal saline within a period of thirty seconds. The Witte peptone caused only a slight augmentation of pancreatic flow (from 4 to 15 drops) and Wilson's meat peptone even less (from 0–10).

In another series of experiments (5 dogs) on anesthetized (pentobarbital sodium) dogs, 10 mgm. per kilo per minute of Witte's peptone in normal saline was injected continuously intravenously for one or two hours. A change in blood pressure did not result or was very slight in

all experiments but one, in which the fall in pressure was marked. After a latent period of about one minute, pancreatic secretion was stimulated, the stimulation being evident over a period of from 20 to 30 minutes and from 25 to 60 drops (2–4 cc.) being obtained (average 45 drops). After the flow ceased, the pancreas was non-responsive or refractory to an intravenous dose of Witte's peptone (30–200 mgm. per kilo) given within 30 seconds or at a slower rate. The rate of injection could be increased to 90 mgm. per kilo per minute without stimulating secretion. Yet, during this refractory state of the pancreas to Witte's peptone, and while the peptone was being continuously injected, the response of the pancreas to a standard dose of secretin was augmented by from 400 to 500 per cent. The refractory state to peptone persisted for at least one-half hour after the continuous peptone injection was stopped, but the control or original response to peptone was immediately restored by a secretin injection. Thus, the secretin injection restored the sensitivity of the pancreas to Witte's peptone. A study of the blood pressure changes failed to show that the development or recovery from refractoriness by the pancreas to Witte's peptone was related to "peptone desensitization."

Five grams of Witte's peptone in 50 cc. of water were injected into the duodenum of two anesthetized and two unanesthetized pancreatic fistula dogs without causing an augmentation of pancreatic flow. About one-half hour later these dogs were given a standard dose of secretin without obtaining the exaggerated response observed above when peptone was continuously injected intravenously. These observations show that peptone is not absorbed in amounts sufficient to augment pancreatic secretion per se, nor in amounts adequate to sensitize the pancreas to secretin. Thus, it is believed that the response shown by the pancreas to an intravenous injection of Witte's peptone is of pharmacologic rather than physiologic significance.

Dextrose. In one experiment a 10 per cent dextrose (C.P. for intravenous use, Pfanstiehl) solution was injected at a rate of 1.5 gram per kilo per hour. Pancreatic secretion was not augmented. Other experiments were not performed because it is known that dextrose in the intestine has no or only a slight stimulating effect upon the pancreas.

Emulsified fats. Three sorts of emulsified fat were employed in the place of the liver extract as used in the foregoing experiments. One consisted of 10 cc. of 50 per cent olive oil finely emulsified (2–4μ) with 2 per cent acacia and 0.5 per cent sodium benzoate diluted to 50 cc. with water. Another consisted of the foregoing emulsion diluted to 25 cc. with a buffer solution to which were then added 25 cc. of fresh pancreatic juice, the mixture then being digested for 24 hours prior to injection. To simulate more normal conditions 50 cc. of fatty (2 to 2.5 per cent fat) chyle collected from the thoracic duct were injected, in some experiments

rapidly, in others slowly. The injections were made over a period of from 20 to 30 minutes, no objective toxic effects being observed. Using the olive oil, experiments were performed on four "acute" and "chronic" pancreatic fistula dogs. Using the fatty chyle, experiments were performed on six "acute" and five "chronic" fistula dogs.

The injection of the olive oil digest caused an appreciable and a more or less sustained fall in blood pressure, and the flow of pancreatic juice was inhibited in the "acute fistula" experiments. In the "chronic fistula" experiments, the flow was not significantly affected. The injection of the fatty chyle had no effect on blood pressure and did not significantly affect the flow of pancreatic juice.

CONCLUSION

These results show that secretin is probably the only humoral agent concerned in the secretory response of the pancreas to a meal.

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THE INFLUENCE OF THE LIVER IN THE FORMATION AND DESTRUCTION OF BILE SALTS

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The metabolism of bile salts is definitely associated with the liver since that organ appears to synthesize and excrete the bile salts of the bile. Only one function is definitely known for bile salts, that of assisting in the digestion of fats. Little is known as to the origin or precursor of cholic acid and little is known as to the products of its destruction in the body. Accurate methods for determination of bile salts in bile have enabled Whipple and his collaborators to draw the following conclusions from their studies of animals with complete biliary fistulas: the daily excretion of bile salts is constant under constant dietary and physical conditions; dietary factors may affect the amount of bile salts formed; bile salts administered by vein or by mouth are rapidly and quantitatively excreted in the bile; the liver is responsible for the elaboration of bile salts; slight injuries to the liver may greatly reduce the amount of bile salts formed and excreted. Sterner, Bartle and Lyon gave a résumé of the literature on metabolism of bile salts.

We have used the method of Gregory and Pascoe with some modifications, so that we have obtained fairly satisfactory results in determination of bile salts in the bile, urine and blood of dogs under various conditions. We have studied formation and destruction of bile salts in normal dogs, in animals with biliary fistulas, in animals with biliary obstruction and in animals following complete removal of the liver.

EXPERIMENTAL PROCEDURE. Bile salts were determined in the blood, urine, and bile by the method of Gregory and Pascoe with certain minor modifications. The method for determination of bile salts in the blood could not be considered as entirely quantitative, but it does appear to be adequate for the determination of the presence or absence of bile salts in the blood and affords a rough estimate of the amount when present. From 1 to 10 cc. of plasma were added to five volumes of cold alcohol and the mixture was centrifuged after chilling for thirty minutes. The precipitate was washed with alcohol and the supernatant fluid evaporated to dryness at 70°C. The residue was dissolved in 20 cc. of alcohol, and

2 cc. of saturated barium hydroxide and 1 cc. of saturated sodium sulphate were added and the mixture centrifuged. The supernatant fluid was evaporated to dryness and taken up in 1 cc. of 0.3 per cent solution of furfural and 6 cc. of 45 per cent sulphuric acid. This was placed in a tube and heated in a water bath at 65°C. for thirty minutes. A series of standards was made at the same time using the same amount of normal serum (which gives no reaction for bile salts) with the addition of 0.1, 0.3, 0.5, 0.8 and 1.0 mgm. of sodium glycocholate. If the final solution was clear, colorimetric comparisons were made but frequently some turbidity persisted so that comparisons were made only by gross matching of the unknown tubes with the standards. The presence of bile pigment did not materially influence the results but when large amounts of bile pigment were present with small amounts of bile salts the determinations were repeated after removal of the bile pigment. This was accomplished by solution of the residue from the alcoholic filtrate in ammoniacal alcohol of pH 10 to 10.5 and addition of "Norite" until the bile pigment was removed. The supernatant fluid was evaporated and the bile salts determined as before.

Urine containing more than 0.5 mgm. of bile salts per cubic centimeter was diluted to approximate this figure and the content of bile salts determined by the Gregory and Pascoe method on 1 cc. of the diluted urine. Some urine contained sufficient pigment to give a measurable absorption of light when heated to 65°C. for thirty minutes with 45 per cent sulphuric acid but without furfural. This reading, against a standard of 0.5 mgm. sodium glycocholate, was subtracted (after allowance for dilution) from that obtained for the content of bile salts of the urine. This method gives fairly accurate results if sufficient bile salts are present. However, with normal urine about 100 mgm. for the twenty-four-hour specimen are obtained which figure probably represents a blank for the determination rather than the actual amount of bile salts, if any are present. We have repeatedly tried to isolate bile salts from normal urine but have not been successful although added bile salts (100 mgm.) may be recovered. For this reason we do not regard figures of less than 100 mgm. in twenty-four hours as being significant.

We found the Gregory and Pascoe method for bile salts in bile to be satisfactory. The presence of bile pigment did not interfere with the determination of bile salts. In most cases considerable dilution of the bile was made for the determination, but even when the content of bile salts was much reduced in proportion to the content of pigment the presence of bile pigment did not interfere with the reaction. Essentially the same values were obtained before and after removal of the bile pigment from the solution.

All surgical procedures were done with aseptic technic and with the

animal under ether anesthesia. Obstructive jaundice was produced by double ligation and section of the common bile duct and the gall bladder was removed at the same time. Complete biliary fistulas were made either by the method of Rous and McMaster or by anastomosis of the common bile duct and the renal pelvis after removal of the gall bladder (3). Complete removal of the liver was accomplished by the three-stage method (2).

Results. The normal dog excretes but traces of bile salts in the urine and bile salts are not definitely detectable, by the methods used, in normal blood. Following intravenous injection of sodium glycocholate or sodium taurocholate (rapid injection of 0.1 gram of bile salts in a 5 per cent solution for each kilogram of body weight) only a small amount appeared in the urine and the injected material rapidly disappeared from the blood. Sodium glycocholate given by mouth in amounts up to 5 grams daily for periods of several weeks did not increase the content of bile salts of the blood, urine, or feces.

After complete removal of the liver, animals were maintained in good condition by repeated injections of glucose in amounts which sufficed to keep the content of glucose of the blood at approximately normal levels. For the first twenty-four hours the volume of urine secreted was usually fairly normal. Only a small nonspecific reaction was given by the Gregory and Pascoe method, which indicated definitely a smaller quantity of bile salts than was present in the urine before the liver had been removed. Usually less than 3 mgm. per hour was indicated by this reaction and all of our efforts definitely to prove the presence of bile salts in this urine have failed, as have similar attempts with normal urine in the presence of a slightly greater concentration of substances which react to the Gregory and Pascoe method. Since our animals were subjected to a period of fasting of eighteen hours before operation reabsorption of bile salts was probably not a factor, as was also indicated by the fact that the small amount of reacting substance appeared to be fairly constant in the urine from hour to hour. We have also been unable to demonstrate the presence of bile salts in the blood following removal of the liver at intervals up to forty-eight hours after operation.

Bile salts (sodium glycocholate or taurocholate) were rapidly excreted in the urine following administration of these substances to animals after complete removal of the liver. In most of our experiments we gave sodium glycocholate by continuous intravenous injection at the rate of 100 mgm. per kilogram of body weight each hour for two hours. In this way 2 to 3 grams of sodium glycocholate were injected. Bile salts immediately began to appear in the urine and approximately 25 per cent was recovered in the urine during the two-hour course of injection; 30 to 40 per cent appeared within the next two hours, 20 to 30 per cent appeared

in the next two hours and 10 to 20 per cent was recovered in the following two hours. After this period there was a rapid diminution in the content of bile salts of the urine so that only the small amount that had been present before injection was found. In all of our uncomplicated experiments 90 to 100 per cent of the sodium glycocholate injected was excreted in the urine within twelve hours after the beginning of the injection. In a few experiments similar results were obtained following injections of solution of sodium taurocholate.

Ligation of the common bile duct and extirpation of the gall bladder of dogs produced definite bilirubinemia and bilirubinuria within six hours after operation. Bile salts were not found in the blood under the conditions of our experiments until from twelve to twenty hours after operation. Within the next twelve hours there was usually a marked rise in the content of bile salts of the blood so that values of 40 to 50 mgm. of bile salts for each 100 cc. of blood were obtained. There was a gradual decline of the content of bile salts of the blood in the next two weeks to a level of about 15 to 20 mgm. per cent, which level was maintained for several months thereafter in uncomplicated experiments. Bile salts were usually definitely present in the urine during the second twelve hours following operation and gradually increased in amount until a definite level was reached on the third or fourth day after operation. The amount of bile salts excreted in the urine thereafter remained fairly constant from day to day for several months. The amount of bile salts excreted was usually about a third to a half of the amount that would be excreted in the bile of a dog of similar size but with a complete biliary fistula.

Bile salts were excreted in the urine following intravenous injection of these substances into animals after ligation of the common bile duct. Injections made, comparable to those given to animals after complete removal of the liver, did not give the quantitative recovery of the injected material that was found when the liver was not present. No essential difference in results was obtained whether the injection was given a few hours after operation or several days thereafter. From 20 to 25 per cent appeared in the urine during the two-hour period of injection, 15 to 25 per cent during the next two hours and about 10 per cent in the next two hours. Only about 10 per cent was recovered within the next twelve hours so that the total recovery of the injected bile salts eighteen hours after injection was about 60 per cent. If allowance was made for the amount of bile salts excreted during this period by animals which had not received injection, the amount actually recovered would be less than 50 per cent of the amount injected. Moderate amounts of bile salts (amounts calculated as the normal daily biliary amount of bile salts excreted by a normal dog of the same size) were administered by mouth daily. The following day an increased elimination of bile salts in the urine

occurred but the total amount was usually less than that given. The amount of bile salts excreted decreased in the next few days until amounts were excreted which were comparable to the level that had been established before administration of bile salts had been begun. Thus, a dog with complete biliary obstruction, which was excreting 500 mgm. of bile salts daily, continued to excrete 500 mgm. daily although 1000 mgm. were given by mouth each day.

Administration of drugs which produce definite hepatic necrosis, such as chloroform, carbon tetrachloride or tetrachlorethane, to animals with complete biliary obstruction, caused marked diminution in the amount of bile salts excreted in the urine. After one hour of anesthesia with chloroform the excretion of bile salts began to decrease on the second day and on the third and fourth days less than half of the expected amount of bile salts was excreted. The content of bile salts of the blood also decreased and in some experiments the blood gave no test for bile salts although considerable amounts were present before, and returned again to the blood after, six or seven days. The excretion of bile salts usually had returned to its former level after about ten days. Similar observations were made following administration by mouth of carbon tetrachloride and tetrachlorethane.

The daily output of bile salts by animals with complete biliary fistula was usually low for the first day or two after operation. After five or six days the daily output became constant at a level of two or three times that excreted in the urine by a similar dog with complete biliary obstruction. Administration of chloroform, carbon tetrachloride or tetrachlorethane to these animals produced a marked decrease in the amount of bile after the second day and greatly reduced the total excretion of bile salts. The concentration of bile salts in the bile was also decreased so that in some cases only traces of bile salts were found in the bile. After several days (depending on the extent of the hepatotoxic action of the drug used) the flow of bile and the content of bile salts returned to normal. In the blood and urine there was no increase in the content of bile salts at any time. When toluylenediamine was given in amounts sufficient to produce jaundice a similar effect on the excretion of bile and bile salts in the bile was noted. However, when this drug was used a marked increase in the content of bile salts of the blood and urine was obtained.

Comment. The formation of bile salts appears to be closely associated with the activity of the hepatic cell. No evidence of formation of bile salts is found in animals following complete removal of the liver, after periods of time in which bile salts are definitely present in the blood and urine of animals following ligation of the common bile duct. The recovery of bile salts after intravenous injection of bile salts into these animals is good evidence that the methods employed were sufficient to recognize

formation of bile salts after hepatectomy if it occurred. Whipple and Smith found that mild hepatic injury caused by chloroform greatly decreased the formation of bile salts. We have confirmed this observation many times and also have found similar effects with carbon tetrachloride and tetrachlorethane. This function of the liver seems specifically injured by these drugs because small amounts which do not produce definite histologic changes or definite alteration in many other functions of the liver produce such marked changes in formation of bile salts. Again, not all drugs which affect the liver, damage the mechanism by which bile salts are formed; for instance, toluylenediamine may cause complete suppression of flow of bile, but the bile salts accumulate in the blood and are excreted in the urine.

That the liver also plays a part in the destruction of bile salts is indicated clearly by the complete recovery of bile salts after their administration to the liverless animal and by the incomplete recovery when similar amounts are given to animals with complete biliary obstruction. The apparent retention of bile salts by the animal whose common bile duct is obstructed might be considered as evidence of retention of these substances by the liver and not of their destruction by the liver, except for the fact that similar results are obtained with animals after the tissues have become saturated with bile salts in the course of the obstructive jaundice. This is further emphasized by the continued disappearance of some bile salts when administration of them is repeated from day to day. It should be noted, however, that evidence of destruction of bile salts in the body is more marked under conditions which increase the content of bile salts of the blood. Bile salts administered to animals with complete biliary fistula are recovered almost completely in the bile, but some loss is indicated if allowance is made for the bile salts that would have been in the bile had none been added by administration. Repeated administration of bile salts gives definite evidence of some destruction of these substances, although less is destroyed than when biliary obstruction is present.

We have also made studies of the destruction of bile salts in animals following toxic injury to the liver, but have been unable definitely to alter the destruction of bile salts, except by total removal of the liver. Animals with biliary obstruction, whose livers also have been damaged by chloroform, excrete slightly less of the injected bile salts but the content of bile salts of the blood remains definitely elevated. Animals with biliary fistula, whose livers also have been damaged sufficiently to prevent the secretion of bile, gave results similar to those with obstructive jaundice and animals that secreted bile gave results similar to those obtained with animals which had biliary fistulas but otherwise were normal.

Should we attempt to transpose our observations on the formation and

destruction of bile salts to the normal animal we would summarize as follows: The normal animal excretes bile salts in the bile in amounts that vary with the diet, approximately 100 mgm. daily for each kilogram of body weight, if all bile is lost as it is with a complete biliary fistula. If excretion of the bile is prevented, as it is if an animal has uncomplicated biliary obstruction, only about 40 mgm. of bile salts for each kilogram of body weight are excreted in the urine. The difference probably represents the capacity for the definite but limited destruction of bile salts under these conditions, but more can be destroyed if additional amounts are administered. Not more than the equivalent of the bile formed in two days can be stored in the gall bladder. Bile salts which reach the intestine in the bile from the liver or gall bladder are rapidly absorbed so that no more than traces are excreted in the feces and little can be destroyed in the intestine since absorption has been shown to be quantitative. Therefore the liver must destroy about half of the bile salts which it makes each day. Unfortunately we have no knowledge as to the disposition of the destroyed material, whether it is synthesized into something that is utilized or whether it is excreted as an unknown waste product. Nor do we know what becomes of the precursors of bile salts which are not formed when the liver is damaged as it is by chloroform.

SUMMARY

The normal dog excretes but traces of bile salts in the urine and bile salts are not definitely detectable in normal blood. Following intravenous injection of glycocholates or taurocholates only a small portion appears in the urine and the injected material rapidly disappears from the blood. After continuous administration of large amounts of bile salts the indication is that destruction of these substances takes place, since only traces are found in the urine and feces.

After complete removal of the liver, bile salts are not found in the blood or urine even after periods of time in which they are readily detected following ligation of the common bile duct. The liver appears to be the site of formation of bile salts.

After complete removal of the liver, injected bile salts are rapidly excreted in the urine so that quantitative recovery of the injected material is usually made within twelve hours. If similar injections are made in animals with biliary obstruction only part of the injected material can be recovered. The liver apparently plays a predominant part in the destruction of bile salts.

Formation of bile salts is inhibited by administration of hepatotoxins such as chloroform, carbon tetrachloride, or tetrachlorethane, but is not affected by other toxins such as toluylenediamine.

Destruction of bile salts is little altered by hepatotoxins that markedly alter the formation of bile salts.

PROTOCOLS

Since all uncomplicated experiments gave similar results, only one specimen of each type is given.

Bile salts appear in the blood and urine following biliary obstruction. Administration of carbon tetrachloride decreases the amount of bile salts in the blood and urine

		TOTAL BILE SALTS IN 24-HOUR URINE
		mgm.
4–25–33		80
	Bile ducts obstructed, ether anesthesia	
4–26–33		110
4–27–33		300
4–28–33		322
4–29–33		946
5– 4–33		1,160
5–30–33		1,136
6–15–33		1,180
6–19–33	Serum: bilirubin 6.5 mgm., bile salts 30 mgm. 10 cc. carbon tetrachloride given by stomach tube	1,060
6–20–33	10 cc. carbon tetrachloride given by stomach tube	725
6–21–33	Serum: bilirubin 7.0 mgm., bile salts 5 mgm.	510
6–22–33		187
6–23–33		220
6–24–33	Serum: bilirubin 7.2 mgm., bile salts 8 mgm.	248
6–28–33		320
6–29–33		650
7– 7–33	Serum: bilirubin 6.5 mgm., bile salts 20 mgm.	1,020
7–12–33		664

The above data were obtained with a male bull terrier which weighed 16.7 kgm. on April 25, 1933. On this date the gall bladder was removed and the common bile duct sectioned and doubly ligated. The animal remained in good condition during the course of this experiment. The animal died August 25, 1933, at which time necropsy confirmed the complete ligation of the common bile duct and nothing was found that would invalidate the results of this experiment.

This animal did not give evidence of definite excretion of bile salts in the urine until the second twenty-four hours after operation. Most animals give evidence of definite bile salts in the urine after the first twelve hours.

Animals with complete biliary fistula excrete more bile salts than those with biliary obstruction. Carbon tetrachloride depresses excretion of bile salts without their accumulation in the blood

		TOTAL BILE SALTS IN 24-HOUR BILE AND URINE
		mgm.
5-12-33		40
	Common bile duct and right ureter anastomosed	
5-13-33		180
5-14-33		3,600
5-15-33		2,820
5-16-33		2,712
6- 1-33		2,356
6-17-33		2,840
6-19-33	10 cc. carbon tetrachloride given by stomach tube	2,500
6-20-33	10 cc. carbon tetrachloride given by stomach tube	1,560
6-21-33	Serum: bilirubin positive, bile salts negative	604
6-22-33	Serum: bilirubin positive, bile salts negative	91
6-23-33		336
6-24-33		1,620
6-25-33		2,025
7-15-33		2,600

The above data were obtained with a female shepherd dog which weighed 16.6 kgm. on May 12, 1933. On this date, under ether anesthesia, the gall bladder and right kidney were removed and the common bile duct anastomosed with the right ureter. At necropsy no signs of jaundice were present and the anastomosis appeared to have been functioning.

Biliary obstruction is followed by a rise in the bile salts content of the blood and urine. There is evidence of destruction of bile salts since they are not completely recovered after injection

		TOTAL BILE SALTS IN URINE
		mgm.
10:00 a.m.	24-hour urine	70
	Bile ducts obstructed, ether anesthesia	
1:00 p.m.	3-hour urine	3
4:00 p.m.	3-hour urine	24
7:30 p.m.	3½-hour urine	32
10:30 p.m.	3-hour urine. Serum: bilirubin positive, bile salts negative	50
9:15 a.m.	10¾-hour urine. Serum: bilirubin positive, bile salts positive	262
2:15 p.m.	5-hour urine. Continuous injection bile salts and glucose begun	110
4:15 p.m.	2-hour urine. Injection bile salts and glucose discontinued	760
7:15 p.m.	3-hour urine	1,050
10:15 p.m.	3-hour urine	210
8:15 a.m.	10-hour urine	251
	2271 mgm. of bile salts excreted in 18 hours following injection of 3560 mgm. of sodium glycocholate	
2–26–33	24-hour urine	556
2–28–33	24-hour urine	610
3– 9–33	24-hour urine	880

The above data were obtained with a male shepherd dog which weighed 17.8 kgm. on February 22, 1933. On this date the gall bladder was removed and the common bile duct was sectioned and ligated. Sodium glycocholate, 3560 mgm. dissolved in 120 cc. of 6 per cent glucose solution, was injected during the two-hour period as indicated. Necropsy was performed on April 19, 1933, at which time the complete biliary obstruction was still present.

Bile salts definitely began to appear in the urine about twelve hours after ligation of the common bile duct and were detected in the blood within twenty-four hours. About 36 per cent of the sodium glycocholate which was injected seems to have been destroyed. Similar results were obtained with animals given injections of bile salts a few hours after operation.

Bile salts are not destroyed in the absence of the liver

		TOTAL BILE SALTS IN URINE
		mgm.
9:00 a.m.	24-hour urine	70
	Liver removed, ether anesthesia	
12:25 p.m.	3-hour urine. Continuous injection of glucose begun	5
2:25 p.m.	2-hour urine	8
4:25 p.m.	2-hour urine	7
6:25 p.m.	2-hour urine. Continuous injection bile salts and glucose begun	7
8:25 p.m.	2-hour urine. Injection bile salts discontinued	582
11:25 p.m.	3-hour urine. Serum: bilirubin positive, bile salts positive	946
1:25 a.m.	2-hour urine	308
6:25 a.m.	5-hour urine. Serum: bilirubin positive, bile salts negative	220
	2056 mgm. bile salts were excreted within twelve hours after the injection of 2080 mgm. of sodium glycocholate	

The above data were obtained with a female bull terrier dog which weighed 10.4 kgm. on February 22, 1933. The two operative procedures preliminary to that of complete hepatectomy had been performed on September 22, 1932 and November 22, 1932. The animal recovered within a few minutes from the effects of the operation of complete removal of the liver and remained in excellent condition throughout the course of the experiment. Continuous injection of glucose (36 cc. of 6 per cent glucose per hour, or 0.2 gram for each kilogram body weight each hour) maintained the level of blood sugar near normal values for the eighteen hours it was administered. Sodium glycocholate, 2080 mgm., was administered in the glucose solution the animal received during the two hours taken for administration of bile salts. At the termination of this experiment the animal was anesthetized and necropsy performed immediately. Nothing was found which might invalidate the results of this experiment.

The small amount of bile salts indicated as being present in the urine before and after removal of the liver probably represents a blank determination and should not be considered as evidence of the presence of bile salts. In other experiments we found similar reactions (2 to 3 mgm. each hour) in the urine as long as forty-eight hours after removal of the liver. No bile salts have been found in the blood after removal of the liver except after they had been administered to the animal.

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REGENERATION OF TASTE BUDS AFTER SUTURE OF THE LINGUAL AND HYPOGLOSSAL NERVES

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It has been shown that cutting the chorda tympani nerve causes degeneration and disappearance of taste buds from the fungiform papillae on the anterior portion of the tongue in the mammal (Olmsted, 1922; Olmsted and Wirtanen, 1934). A similar disappearance of taste buds from the barbels of the catfish follows the cutting of the appropriate branch of the VII cranial nerve, and in this animal regeneration of taste buds and restoration of amputated ends of barbels take place when the nerve again grows down into the barbel (Olmsted, 1920).

The following work has been undertaken to determine whether the presence of the VII nerve is specific for the regeneration of taste buds or whether another cranial nerve not concerned with the sense of taste can, upon growing into the denervated region, serve as a stimulus for the differentiation of epithelial cells into the special gustatory cells.

In one series of dogs the lingual nerve was divided on one side at the point where it emerges from under the jaw making a sharp angle before it runs forward beneath the lower surface of the tongue. This series served as a control since in these animals the two ends of the divided nerve were brought together and sutured. Histological preparations of portions of the anterior region of the tongue containing fungiform papillae were made. The tissue was fixed in Zenker-formol, stained either with Mallory-Azan, or with iron-hematoxylin, using as counter stain aniline-blue according to an unpublished formula furnished through the courtesy of Dr. Alexis Koneff. Normally there are four to five taste buds to a single fungiform papilla on the anterior part of the dog's tongue. Certain of these control dogs were killed one month after the operation and the histological preparations of the papillae showed entire absence of taste buds in the denervated area. This was to be expected if the regenerating nerve had not had time to reach the papillae (Olmsted, 1920). When these control dogs were killed four to five months after the operation, it was found that regenerated nerve fibers could be traced into the papillae

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and that taste buds were now present. In one dog, out of twenty papillae picked at random, one was without taste buds, six had one each, the other thirteen had two to six each. In another dog there was an extraordinary number of regenerated taste buds, ninety-nine in thirteen papillae, three papillae each having ten taste buds, double the normal number.

In the dog, therefore, as in the catfish, growth of nerve fibers from the original nerve stump into the papillae is followed by regeneration of taste buds.

In another series, both the lingual and hypoglossal nerves were divided. The peripheral end of the lingual was sutured to the central end of the hypoglossal and vice versa. In these dogs the denervated side of the tongue became somewhat atrophied and the border was scarred. This half of the tongue being insensitive failed to initiate protective reflexes. It was found on autopsy that growth from the raw ends of the divided nerves formed a dense knot of tissue through which it was impossible to trace the course of the nerve fibers. In one of these dogs, sacrificed four months after operation, there were no regenerated taste buds; in another dog, eight months after operation, single taste buds were found in two papillae, two in a third, while four papillae were entirely without taste buds. Since it was not perfectly clear that some of the fibers from the central end of the lingual might not have found their way to the papillae, it was decided to modify the operation.

The lingual nerve was divided near the jaw and the central end cut back as far as possible; the hypoglossal was divided at a point where the peripheral end of the lingual could be attached to the central end of the hypoglossal without stretching. A section of the peripheral end of the hypoglossal was cut away so that the raw end of neither of the two nerves was within a centimeter of the suture. Five to six months later, stimulation of the hypoglossal a short distance central to the suture caused wrinkling of half the tongue except at its tip, whereas stimulation peripheral to the suture produced no movements.

Five months was evidently too short a time to allow for regeneration. In one dog, thirty papillae were examined after this interval and only one fully developed taste bud was found. However, the nerve branches could be traced into the papillae and several papillae showed evidence of beginning regeneration of taste cells. This evidence was based on the presence of a greater number of mitoses than usual in the inner row of epithelial cells, and the appearance of pear-shaped clusters of cells showing a tendency to elongate in a direction which would eventually bring their ends to the surface of the papilla. A second dog had one to two perfect taste buds in each of three papillae, none in seven others.

Two dogs were kept six months. In one, out of seventeen papillae three had no taste buds; fourteen had a total of fifty-nine, an average of

four each, the normal number. In the other dog, nine papillae were examined. All had taste buds, two having one each, the others three to five each. A statistical count of the number of fibers in the hypoglossal just central to the suture and in what was originally the peripheral part of the lingual distal to the suture was kindly made for us by Mr. Kazuo Yanagisawa. He found that sixty-five per cent of the fibers from the hypoglossal had found their way into the peripheral remains of the lingual.

These experiments show that it is not the presence of the VII cranial nerve specifically which initiates the return of taste buds in papillae where they have disappeared as a result of denervation; growth of fibers from the XII, which is essentially a motor nerve, can also provide a stimulus for differentiation of taste cells. The XII, although motor, of course contains a certain proportion of sensory fibers to provide for proprioceptive impulses from the tongue muscles. These experiments do not, therefore, make it possible to state definitely whether only sensory, or whether motor fibers as well, can bring about this regeneration of taste cells. In the XII nerve, however, the proportion of sensory fibers is evidently rather small, and because in these last two dogs so many papillae not only had taste buds but had their normal quota, the presumption is very strong that motor fibers as well as sensory fibers cause the taste buds to reappear.

CONCLUSION

The XII cranial nerve, essentially motor, when sutured to the lingual, can initiate regeneration of taste buds which have disappeared from fungiform papillae on the tongue of the dog as a result of cutting the lingual nerves.

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FUSILLADE PATTERNS OF INSPIRATORY AND EXPIRATORY MUSCLES AND THEIR EFFECTS ON THE RESPIRATORY ACT[1]

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Our studies on the action potentials of the inspiratory and expiratory muscles of the dog, during eupnea, have shown such frequent characteristic differences that they seem worthy of description, especially since they may have important bearings on the mechanics and the nervous control of breathing.

RESULTS. *Fusillade patterns of inspiratory muscles.* At the 1933 meetings of the Physiological Society we reported on two points relating to our present subject (Gesell, 1933), namely, the acceleration of fiber rhythm, and recruitment of muscle fibers during inspiratory contractions. When closely approximated bipolar electrodes were applied to a relatively inactive portion of an inspiratory muscle, such as the diaphragm or intercostal muscles, the potentials were usually discrete and showed an initial fiber rhythm of approximately 5 per second, accelerating progressively up to the height of inspiration, to a final rate of 10 to 15 per second. (See fig. 1, Gesell, 1936, and fig. 1 of this paper.) The acceleration and final fiber rhythm varied considerably with the dog and with the type of breathing which prevailed. A slowly breathing animal might show a moderate acceleration while a rapidly breathing animal might exhibit greater acceleration, particularly if the depth of breathing was also augmented. Deacceleration of fiber rhythm at the end of inspiration was decidedly abrupt. Such changes in fundamental fiber rhythm were more or less characteristic of inspiratory contractions.

The second point was demonstrated to best advantage when widely separated electrodes were employed or when closely approximated electrodes were moved to more actively contracting muscle (see fig. 1, Gesell, 1936, and fig. 1 of this paper). Then, in addition to the acceleration of fiber rhythm, new potentials added themselves to the electrogram in ever increasing numbers, up to the point of expiration when the process was suddenly reversed. The results conform with the early description of

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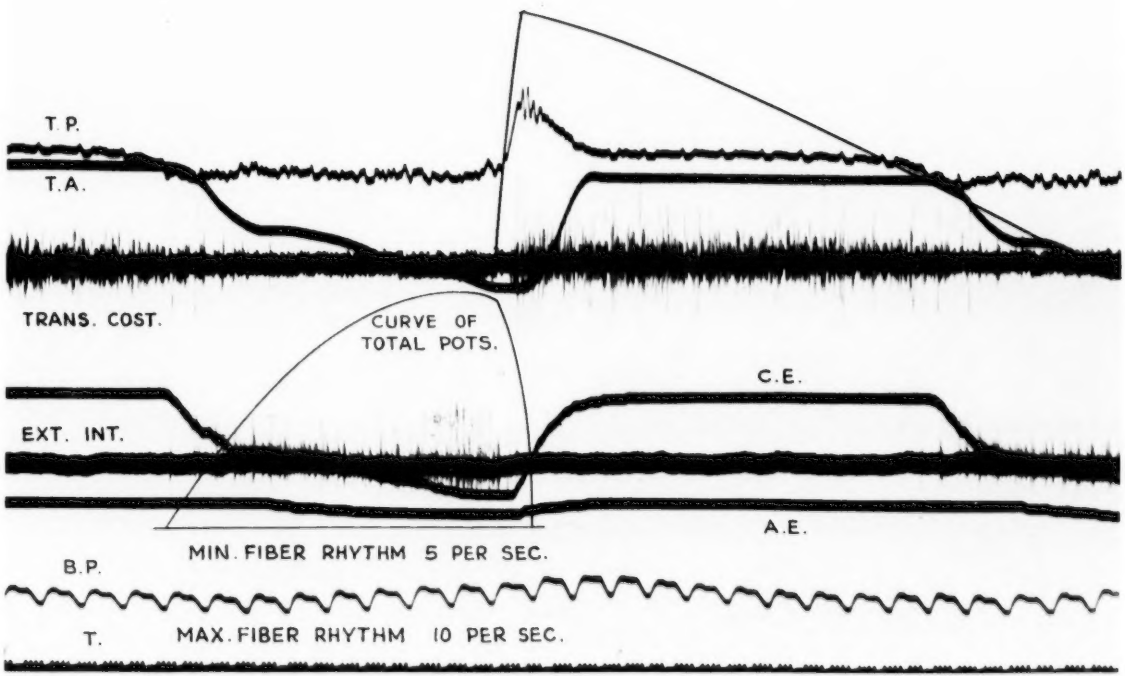
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T.A.
TRANS. COST.
CURVE OF
TOTAL POTS.
C.E.
EXT. INT.
A.E.
MIN. FIBER RHYTHM 5 PER SEC.
B.P.
T.
MAX. FIBER RHYTHM 10 PER SEC.

Fig. 1

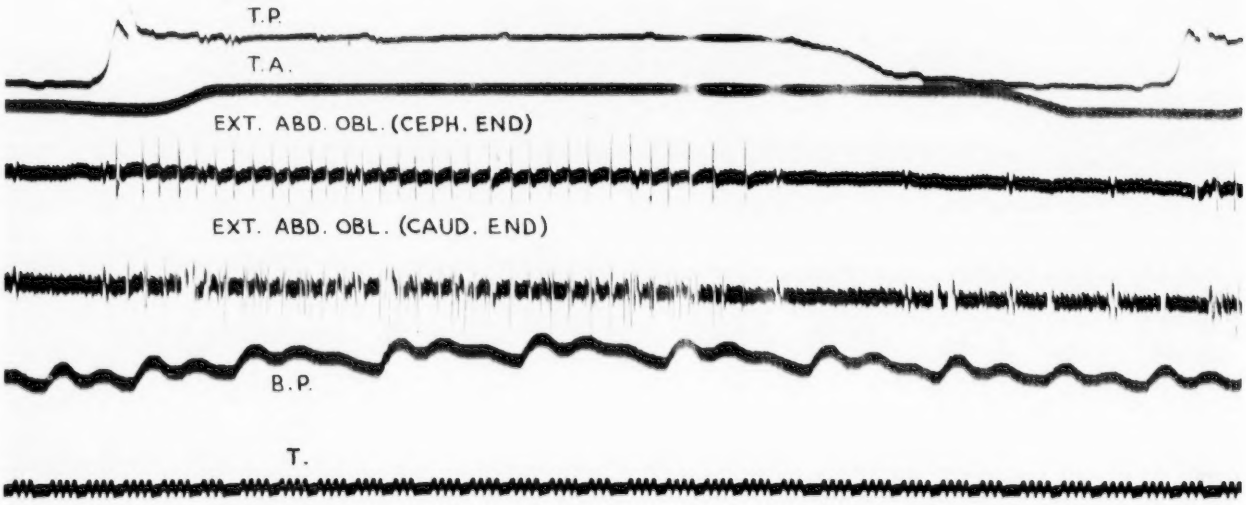
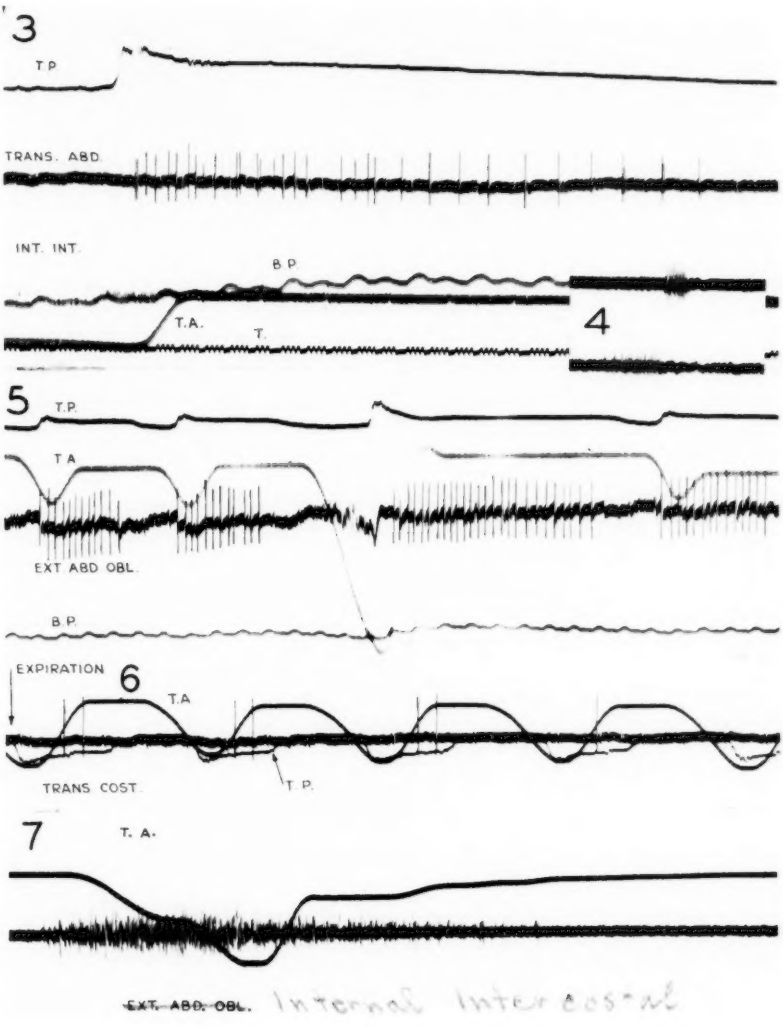


Fig. 2



Figs. 3-7

Figs. 1 to 7. Electrograms of various muscles. For description of results see text. *T.P.*, tracheal pressure, downstroke represents inspiration. *T.A.*, tidal air recorded with a spirometer, downstroke represents inspiration. Due to the inertia of the spirometer the time relations of the changing phases of breathing are not as accurately recorded as in the *T.P.* record. *B.P.*, blood pressure and pulse. *T.*, time in 0.04, 0.20 and 1.0 second intervals.

the contraction of diaphragmatic slips by Head (1889). They are comparable to the findings of Adrian and Bronk (1929) on the frequency discharge of reflex and voluntary contractions and the recent results of Bronk and Ferguson (1935) but differ from the findings of Adrian and Bronk (1928) on the phrenic nerve in which acceleration and recruitment are described as missing.

The accession of potentials during the inspiratory act, resulting from recruitment and accelerated rhythm, produced a more or less characteristic triangular appearance of the fusillade, with the apex at the left corresponding with the onset of inspiration (see figs. 1 and 4). Where the potentials are sparse, this appearance may not be so obvious, but under these conditions the triangular nature of the fusillade is brought clearly into evidence by plotting total number of potentials against time, as was done in figure 1.

Fusillade patterns of expiratory muscles. Expiratory activity differed from the inspiratory activity in characteristic ways depending on the mode of breathing which prevailed. Three types of eupnea were encountered. Breathing in which no participation of expiratory muscles occurred; breathing in which the contractions were of a tonic nature, the number of active fibers and the rhythm of individual fibers remaining relatively uniform throughout the expiratory phase; and breathing in which expiratory contractions were initiated by a burst of potentials decreasing in number as expiration progressed.

The first type, with expiration entirely passive was relatively uncommon. The second and third types were common. Figure 2, showing fusillade patterns of the M. obliquus abdominis externus with one electrode set on the cephalic half for discrete potentials and the other, on the caudal half, for indiscrete potentials is an example of the second type. Ignoring the momentary acceleration at the onset and the momentary deacceleration at the end of expiration, fiber rhythm maintained an exceptionally uniform tempo producing a characteristic "rectangular" contraction. The third type, contrasted with its accompanying inspiratory contraction, is illustrated in figures 1 and 4 showing a reversed "triangular" configuration. Decline of potentials during expiration, like incline during inspiration, varied markedly from one animal or one condition to another. In figure 4 the decline was very abrupt; in figures 1 and 3 where breathing was slow and expiration prolonged, the diminution of potentials was very gradual and began early in the phase of expiration. Under such conditions some fibers may contract only for the moment while others persist up to the very end of expiration as seen in figure 3.

The mechanism of the reversed configuration of inspiratory and expiratory fusillades is a matter requiring further study. So too are the mechanisms underlying the three types of eupneic breathing. Obviously breathing which involves only the use of inspiratory muscles is a simpler and

different nervous procedure than breathing involving the alternate use of two antagonistic groups with reciprocal inhibition, timed to eliminate antagonistic waste of energy. But whether breathing of type two differs fundamentally from that of type three is not so apparent. If the rectangular contractions are tonic contractions of reflex origin they cannot be considered a result of direct discharges from the so-called expiratory center, but rather a result of an innervation of an entirely different nature, yet giving all outward indication of expiratory discharges. That the respiratory center is capable of influencing various spinal reflexes is well known, and the idea of inhibition of tonic activity of expiratory muscles during the phase of inspiration is not new. Starling (1935) refers to this phenomenon in discussing the probabilities of active and passive expiration during eupnea. "It seems possible that the more or less muscular activity of expiratory muscles is alternated with that of the inspiratory muscles. In fact Sherrington's results on the coördination of muscular movements would lead us to assume inhibition of the tone, e.g., of the abdominal muscles during inspiration and an active augmentation of their tone during expiration." In our experiments, rectangular expiratory contractions give all indications of resulting from the release from rhythmic inhibition of a continuously driven tonic reflex, the inhibitions coinciding with the inspiratory phases of breathing.

This is further supported by results occasionally observed on sporadic deep breaths (see fig. 5). Though the sporadic deep inspiration was exceptionally large and accompanied by a proportionately increased expiratory excursion, the expiratory fusillade, excepting for its increase in length, was in no way augmented over that of the preceding fusillades. Unless other expiratory activity was increased, we must conclude that, despite the presence of expiratory potentials in the M. obliquus externus abdominis, the extra-expiratory excursion was primarily a passive phenomenon. The inspiratory act may, therefore, be strengthened without augmentation of the associated expiratory act. This favors the view that ostensibly active expiration may be nothing more than the resumption of viscero-postural reflexes temporarily inhibited during the phase of inspiration.

If we inquire into the nature of tonic contractions which differentiates them from clonic contractions, lengthy duration and uniformity of contraction and reflex origin seem to be the most characteristic attributes. Certainly the uniform rate and number of potentials in figure 2 would classify the contraction as tonic even though the relatively short duration might not. On the other hand contractions of this type may be exceptionally long in very slowly breathing animals during barbital anesthesia and administration of pure oxygen. While the phases of inspiration were of normal duration the expiratory pauses lasted in some instances three

minutes or more, during which time the expiratory muscles were continually and uniformly active. Inhibition occurred only long enough to facilitate the inspiratory filling of the lungs. Death may occur during such extended expiratory pauses, with expiratory muscles contracting up to the end and for a much longer period than that just described. If such protracted expiratory activity and that of figure 2 may be classified as tonic contractions, then shorter expirations which give all the appearance of clonic contractions may also be so classified. See figure 6 which shows contractions of only 1 or 2 twitches.

Source and rôle of tonic expiratory contractions. Theoretically, tonic contractions of the abdominal walls may arise reflexly from distending forces or tugging on visceral suspensions and have for their primary function the maintenance of proper visceral posture. Momentary inhibition of these tonic contractions would favor inspiration and the return of contractions would favor expiration and in that sense the alternate activity and inactivity of the abdominal muscles might be considered an integral part of the respiratory act, even though primarily controlled for the purposes of visceral posture. This reasoning is supported by the reflex contractions which were elicited in quiescent abdominal muscles by an increase of intra-abdominal pressure. In such artificially provoked, as well as in unprovoked abdominal tonus, contractions became rhythmic through rhythmic inhibitions during inspiration. Similar response of expiratory muscles to stretching was invariably seen in the completely resting transcostar muscle when weights were attached to the freed tendinous insertion. The strength of contraction, as indicated by the number and rate of action potentials, varied with the stretching weight and once such a contraction was initiated it was rhythmically interrupted with every inspiration.

Such important consequences of proprioceptive reflexes and their rhythmic interruptions suggest mechanisms of detailed guidance of the respiratory act. For example, is it possible that the stretch on the proprioceptive end organs varies in different muscles due either to peculiar mechanical conditions prevailing at the time or due to variations in differential growth of muscle and of the sensory end organs? It is generally accepted that the elastic pull of the lungs which produces the negative intra-thoracic pressure is due to a difference in rate of growth of the lungs and the surrounding thoracic cage. A difference in rate of growth of lung tissue and its proprioceptive endings in the lungs is a comparable idea and it is known that these endings are continually discharging even at the end of the expiratory pause.

By a systematic mapping of potentials we have found no two animals to breathe alike (Gesell, 1936). This "individuality" of breathing shows itself in varying combinations of muscles participating; in variations in

strength of contraction of individual muscles; and in variations of sequence of muscle action. These variations are very striking. One animal may use inspiratory muscles exclusively. Another animal may use every important expiratory muscle. Still others will use varying numbers and varying combinations of expiratory muscles. Could an inherent difference in the degree of the stretch of receptors in various respiratory muscles possibly account for this selective use of respiratory muscles in the respiratory act? Could it account for differences in muscle sequence so commonly observed in experiments on the dog? These questions are put primarily as hypothetical speculation and with little direct experimental support other than that already mentioned. But we beg to mention that we have observed activity of the M. obliquus externus abdominis not infrequently restricted to a narrow band which seemed to be "stretched" over the lower costal borders.

Mechanical and other consequences of fusillade patterns. Differences of fusillade patterns of inspiratory and expiratory muscles must have important mechanical effects upon the act of pulmonary ventilation. When expiration is passive, all of the energy is provided by the inspiratory group of muscles, the chest expands, the diaphragm descends, the abdominal walls bulge to give way to the expanding lungs, all of which automatically increases the counter elastic pull opposing the inspiratory muscles thus calling for ever increasing strength of muscular contraction if inspiration is to continue. The triangular fusillade, which is indicative of progressively augmenting contraction, fits perfectly with the mechanical requirements of the inspiratory act, and the result is a smoothness of mechanical effect not attainable in a rectangular contraction when full energy is released at the onset of the inspiratory act at a time when resistance or counter pull is low.

In this respect the mechanics of expiration differ rather strikingly from those of inspiration in all three types of breathing described above. When the expiratory act is passive, its progress depends essentially on the mode of denervation of the inspiratory muscles and the dissipation of potential energy thus released. At the end of inspiration the torso is maximally stretched and the sudden denervation of the inspiratory muscles introduces a powerful though passive compression of the lungs, the force of which progressively diminishes as the potential energy is spent. The expiratory puff, as witnessed by the sudden rise of intrapulmonary pressure and sudden deflation of the lungs indicates the nature of the mechanical forces at work.

The close dependence of the expiratory act upon the mode of denervation of the inspiratory muscles is clearly indicated in those instances in which inspiratory denervation deviates from the sudden single stage denervation which usually prevails. In figure 7 denervation occurred in

two distinct stages. Each stage produced its effects upon the expiratory act. The initial stage was accompanied by the usual expiratory puff and a partial emptying of the lungs but the final deflation was only slowly accomplished as the inspiratory potentials faded from the record.

Rectangular and triangular expiratory contractions accentuate the mechanical conditions of passive expiration. Powerful active compression is added to the initially strong passive compression and both fade as expiration continues.

Just what is gained by the mechanics peculiar to expiration is difficult to estimate but a phenomenon, not infrequent with moderate anesthesia, which has long excited our curiosity now seems to receive a partial explanation. I refer to fluctuations of the volume of the lungs at the close of inspiration which are sometimes gross as compared with the more uniform volume at the close of expiration (Nielson and Roth, 1929). When we stop to consider the forces at work controlling the progress and extent of the inspiratory act we find two well known opposing processes. Each act of inspiration is the result of an increasing discharge of the respiratory center involving increasing nerve units, discharging at increasing rates. This discharge, according to theories of nervous control of breathing (Hering and Breuer, 1868; Gad, 1880; Head, 1889; Adrian, 1933) is brought to a close by afferent impulses, self generated in the endings of the vagus nerves by the inspiratory act. The experiments of Gesell and Moyer (1935), Brookhart, Steffensen, and Gesell (1935) showing that inhibition of breathing varies directly as the lung volume, and the findings of Adrian (1933) demonstrating a linear relation of proprioreceptor discharge to lung inflation show the crescendo nature of the process counteracting the inspiratory discharge. This simultaneous competition for supremacy by two rapidly, but perhaps not evenly augmenting forces, we believe provides conditions for the uneven inspirations. On the other hand, the waning forces of expiration oppose increasing resistance and, therefore, come into uniform equilibria at the close of expirations.

Since active stretch of the lungs during the phase of inspiration checks inspiration and tends to speed up breathing, and since protracted inflation prolongs expiration and tends to slow breathing, the significance of any influence controlling lung volume is self-evident. It is, therefore, suggested that the mechanics of expiration which determine the protracted inflation of the lungs is a potent factor in the stabilization of the rate of breathing.

SUMMARY AND CONCLUSIONS

1. Fusillade patterns of inspiratory and expiratory contractions during eupnea were compared and their mechanical effects considered.

2. Inspiratory contractions were characterized by a *progressively accelerating* rate of muscle fiber twitching and by a *progressive recruitment*

of fibers up to the end of inspiration. Expiration was initiated by a *sudden* reversal of this inspiratory innervation. This mode of innervation and denervation produced a "triangular" configuration of the electrogram indicating the changing strength of muscular contraction.

3. Expirations were of three types: 1, passive; 2, "rectangular"; 3, "triangular." Rectangular expiration was characterized by a uniform muscle fiber rhythm and by a uniform number of action potentials. Triangular expiration was characterized by a maximum number of potentials at the *beginning* of expiration *progressively diminishing* with time.

4. It is suggested that "rectangular" and "triangular" expirations may be fundamentally different: that triangular expiration is a product of a direct discharge from the expiratory side of the respiratory mechanism; that rectangular expiration may be the resumption of a continuously driven tonic viscero-postural reflex temporarily inhibited during the phase of inspiration.

5. This conception is supported by experiments on the transcostar muscle. Reflex contraction elicited by weighting the free end was rhythmically interrupted with each inspiration, giving all outward appearances of active expiratory contractions.

6. A sporadic deep breath which enormously augments the inspiratory fusillade may have no effect upon the succeeding expiratory fusillade, though the expiratory excursion is correspondingly increased. This shows that inspiratory and expiratory innervations may be separately modified and supports item 4.

7. It is proposed that proprioceptive reflexes may contribute towards the guidance of the respiratory act by influencing the incidence, strength and sequence of contraction of respiratory muscles.

8. Waxing inspiration meets increasing resistance and, therefore, tends to produce a mechanically smooth inspiratory act, but the quickness of development of the counter force of the vagal proprioreceptor inhibitory impulses tends to check inspiration at slightly different levels and produce uneven filling of the lungs.

9. The sudden liberation of expiratory forces at the beginning of expiration tends toward jerky initiation of expirations, but waning mechanical forces meeting increasing resistance tend to come into uniform equilibria at the close of expiration and produce a characteristically even emptying of the lungs.

10. These mechanisms controlling lung volume are suggested as factors influencing the rate of breathing.

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SOME PHYSICAL AND PHYSIOLOGIC PROPERTIES OF THE SYSTEM INSULIN-TANNIC ACID

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It was originally shown in this laboratory (1) (2) (3) that the qualitative and quantitative responses of the pituitary gonadotropic extracts and of insulin could be modified by administering such extracts in combination with various protein precipitants and adsorbents. That the effects produced were attributable to delayed resorption was demonstrated by the production of similar effects by divided dosage. Confirmations of these findings in regard to insulin have now appeared (4) (5). One group of workers (using zinc, the precipitant originally reported by Maxwell) is, however, unwilling to accept the explanation of the phenomenon offered by us, and the other group, apparently overlooking our findings, has limited itself to one hormone and one class of protein precipitants.

Since the principle involved is widely applicable to both theoretical and practical problems, a more thorough investigation of the phase relating to insulin has been made. The present paper summarizes results in the use of tannic acid to produce delayed resorption of insulin.

The conditions and stoichiometric relations under which insulin is precipitated by tannic acid were determined. Physiologic experiments were made in which the effect of excess tannic acid, the factors of sensitivity and resistance to insulin, the level of the blood sugar, and the effect upon incidence of insulin shock were investigated.

Combination of insulin and tannic acid. Tannic acid precipitates insulin on both sides of the isoelectric point and shifts the point of solution approximately to pH 7.0. In a solution of low electrolyte concentration insulin tannate forms a colloidal solution. The colloid is broken by the addition of NaCl. (Tannic acid in a concentration of 1 per cent is not precipitated by NaCl below 20 per cent concentration.)

Crystalline insulin and tannic acid were taken up in 1 per cent NaCl solutions so that 1 mgm. per cubic centimeter of the former and 3 mgm. per cubic centimeter of the latter were contained in solution. Equal volumes of the solutions were mixed and a control solution of insulin plus an equal volume of 1 per cent NaCl was mixed. By means of N/100 NaOH and M/100 HCl in 1 per cent NaCl the pH was adjusted to the point at which the precipitates dissolved and the point at which the precip-

itates formed. With brom thymol blue and phenol red as indicators it was found that crystalline insulin was completely soluble at pH 6.2, the tannate still insoluble at 7.0. From the alkaline side, the tannate became insoluble at pH 7.0, the control at pH 6.0.

Commercial insulin was taken to the isoeletric point, the precipitate removed by centrifugation, washed twice with water and dried (15.1 per cent N). To 180 units of this preparation in 3 cc. H_2O (pH 4.7) were added 100 mgm. tannic acid in 1 cc. H_2O. The precipitate was removed by centrifugation, washed twice with water and dried (8.3 per cent N).

$$\text{Ratio } \frac{\text{insulin}}{\text{tannic acid}} = \frac{5.5}{4.5}$$

Twenty milligrams of crystalline insulin were dissolved in water at the acid side of the isoelectric point in 5 cc. volume. To the solution were added 5 cc. of a solution containing 50 mgm. tannic acid and 200 mgm. NaCl. The precipitate was removed by centrifugation in a 15 cc. centrifuge tube, washed with 10 cc. 4 per cent NaCl. After centrifugation the tube was allowed to drain and was dried with filter paper. The precipitate was dried (9.1 per cent N, 0.5 per cent ash).

$$\text{Ratio } \frac{\text{insulin}}{\text{tannic acid}} = \frac{6}{4}$$

A higher ratio of tannic acid was obtained in one preparation (unpublished data of Maxwell and Bischoff). Two hundred milligrams of tannic acid were added to 400 units commercial insulin in 10 cc. volume. The precipitate was washed once (6.3 per cent N).

Influence of tannic acid upon the incidence of insulin shock (rats). Young 100 to 200 gram rats were separated into two groups by distribution of litter mates. One group received a dose of insulin (2, 3, 4, or 6 units per kilo) mixed in the syringe with an equal volume of water. The other group received the same amount of insulin mixed with a tannate solution corresponding to 10 mgm. tannic acid per kilo. The rats were used only once to avoid the possibility of a change in insulin tolerance, so that each dosage level was ascertained upon a new group of rats. The animals were considered to be in insulin shock when they manifested typical insulin convulsions or when they were prostrated (unable to turn over when placed on the back). The control rats in the series given 6 units per kilo were given tannic acid at a site removed from that of the insulin injection, as a control procedure for the possible effect of tannic acid alone.

The results are clear cut and striking and indicate that the potency of insulin is increased over 100 per cent when administered with tannic acid

and assayed by the establishment of a frequency curve of dosage against incidence of shock (table 1).

Influence of insulin tannate upon blood sugar response of rabbits. Experiment I. *At hyperglycemia.* Eight rabbits were studied once weekly following a 24 hour fast. They were subjected to the following tests, the

TABLE 1

Augmentation of physiologic effect of insulin by administration as the tannate—measured as incidence of insulin shock in young rats

NUMBER OF RATS	UNITS PER KILO	INCIDENCE OF INSULIN SHOCK	
		Control	Tannate dosed (10 mgm. per K)
		per cent	*per cent*
36	2	0	0
26	3	0	23
39	4	0	30
35	6	12	78

order of the tests being reversed to lessen the factor of a possible change in tolerance:

a, glucose tolerance
b, glucose tolerance + insulin
c, glucose tolerance + insulin and tannic acid
d, glucose tolerance + insulin and an excess of tannic acid
e, glucose tolerance + insulin and colloidal iron hydroxide
f, repetition of one of the above tests

The glucose was given subcutaneously The insulin was given at the same time intramuscularly with or without the protein precipitants. The quantities of substances administered and the blood sugar values are given in table 2. The results for the iron have not been tabulated as they paralleled those for the excess tannic acid.

The mean blood sugar values are given with their standard deviations of the mean. The latter should not be used in comparing the different tests since the same rabbits were used in each successive test. They have been calculated to show the degree of variability of the values.

The results (table 2) at the first hour are clear cut and demonstrate the marked delay in the liberation of the insulin when given with tannic acid, a larger excess of tannic acid producing a more profound delay. At the fifth hour the effect of the regular insulin is wearing off, while the insulin-tannate (excess) combination is producing its maximum effect.

Experiment II. *Rabbits sensitized to insulin.* The purpose of this experiment was to study the effect of delayed insulin liberation in rabbits

which had become sensitized to insulin hypoglycemia. In order to eliminate the factor of resistance change each test was repeated on alternate weeks and the values rejected if a change in response was noted. Eight rabbits which had been used repeatedly for insulin studies were selected. They received on successive weeks following a 24 hour fast:

a, 0.5 unit insulin per kilo intramuscularly
b, 0.5 unit insulin plus 1 mgm. tannic acid intramuscularly
c, repetition of *a*
d, repetition of *b*

The values reported in table 2 are the means for six pairs of rabbits, which showed no change in tolerance, during the period of three tests.

TABLE 2

Influence of insulin tannate upon the blood sugar response of rabbits

	GLUCOSE PER KILO	INSULIN PER KILO	TANNIC ACID	BLOOD SUGAR			
				1 hour	3 hours	5 hours	7 hours
	grams		*mgm. per kgm.*				
Expt. I:							
8 rabbits	3.0			267 ±14	192 ±19	121 ±4	128 ±4
Same	3.0	2.0		128 ±20	74 ±04	87 ±6	102 ±6
Same	3.0	2.0	2.5	266 ±15	98 ±08	75 ±4	86 ±6
Same	3.0	2.0	0.35	200 ±20	76 ±03	81 ±4	95 ±6
Expt. II:							
6 rabbits		0.5		60 ±05	70 ±08	103 ±4	
Same		0.5	1.0	87 ±04	65 ±04	72 ±4	
Expt. III:							
7 rabbits		1.0		52 ±05	86 ±05	110 ±3	
8 rabbits		1.0	1.0	63 ±04	63 ±06	91 ±8	
Expt. IV:							
7 rabbits		1.0		59 ±03	53 ±04	83 ±6	
Same		1.0	0.06	59 ±03	53 ±03	67 ±6	

Differences in sugar values at the first and fifth hours are highly significant, and illustrate the effect of the protein precipitant in delaying the insulin action.

Experiment III. *Resistant rabbits.* The purpose of this experiment was to study the effect of delayed insulin resorption in rabbits which had not been sensitized to insulin. Twenty rabbits were divided into two groups at random. After a 24 hour fast, one group received 1 unit insulin per kilo intramuscularly, the other group 1 unit insulin with 1 mgm. tannic

acid. Three rabbits in the control and two rabbits in the tannate-dosed group had insulin convulsions and were eliminated from the experiment. The results are tabulated in table 2. An augmentation in the physiologic effect of the insulin is indicated.

Experiment IV. In this experiment the amount of tannic acid used approaches the theoretical amount which combines with insulin. Blood sugar values the first and third hours show no significant difference. The fifth hour the blood sugar of the tannate dosed rabbits is significantly less than that of the controls ($p = 0.01$). Here again the standard deviation of the mean is given in table 2 to show the degree of variation for the group, but should not be used to test significance, as the same rabbits were used for both experiments. Fisher's formula for the mean of a unique sample was used for testing significance.

Discussion. A lengthy discussion of the results appears unnecessary, as they are clear cut and self explanatory and extend our original findings. It is evident that tannic acid, by decreasing the solubility of insulin and surrounding proteins, prolongs the liberation of insulin given intramuscularly, the prolongation being increased by tannic acid in excess of the amount necessary to combine with the insulin. In the post-absorptive state the prolongation of liberation results in an apparent augmentation of physiologic response as measured by shock (in rats), and by blood sugar changes (in rabbits). During the absorptive stage no augmentation effect is indicated, and in rabbits sensitized to insulin the augmentation is less marked. Since the mechanism of insulin action is still a mystery, the criteria of shock and of prolongation of hypoglycemia may, it will be admitted, not be true measures of the insulin action. The expression augmentation is therefore used advisedly.

SUMMARY

1. Tannic acid precipitates insulin, the pH of solution being approximately 7.0. In low electrolyte concentration a colloid forms.

2. By administering insulin parenterally in combination with tannic acid a prolongation of resorption is effected, producing an augmentation of physiologic effect under certain conditions.

3. The augmentation effect was demonstrated by establishing a frequency curve for incidence of shock against insulin dose (136 rats) and by blood sugar curves in rabbits in the post absorptive state.

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